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EFFECTS OF METHYLPREDNISOLONE ON MYOCARDIAL INJURY AND TECHNETIUM-99m PYROPHOSPHATE DISTRIBUTION AND EXCRETION FOLLOWING TRANSTHORACIC DC COUNTERSHOCK

RICKY MARC SCHNEIDER

1977

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EFFECTS OF METHYLPREDNISOLONE ON MYOCARDIAL INJURY AND TECHNETIUM-99m PYROPHOSPHATE DISTRIBUTION AND EXCRETION FOLLOWING TRANSTHORACIC DC COUNTERSHOCK

RICKY MARC SCHNEIDER

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DEDICATED TO

WENDY, MY FIRST LOVE

MY MOTHER AND FATHER

MY SISTERS, JILL AND WENDY

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INTRODUCTION

Transthoracic direct current countershock causes myocardial necrosis in dogs with preservation of normal regional myocardial blood flow. Technetium - 99m stannous pyrophosphate, a myocardial infarct imaging agent, depends on blood flow for delivery to tissue. Countershock injury, unlike infarction, should permit myocardial radionuclide uptake in direct proportion to the extent of tissue necrosis.

Experiments were undertaken to validate the quantitative use of ^{99m}Tc stannous pyrophosphate in the countershock model by correlation with an independent indicator of myocardial injury, tissue creatine phosphokinase depletion. This model was then used to assess the effects of pretreatment with methylprednisolone, a corticosteroid which has been reported to protect against myocardial infarction. Precordial electrocardiographic ST segment mapping and tissue histologic and histochemical studies served as additional means of assessing myocardial damage. Further experiments evaluated effects of methylprednisolone on ^{99m}Tc stannous pyrophosphate distribution and excretion.

REVIEW OF THE LITERATURE

DC Countershock and Myocardial Injury

Direct current (DC) countershock was introduced almost 15 years ago, supplanting alternating current (AC) defibrillation in the treatment of certain cardiac arrhythmias resistant to drug therapy and requiring urgent conversion. DC countershock has rarely been associated with hypotension, left ventricular failure, and systemic and pulmonary embolism (1, 2), but the most frequent complications in man and in experimental animals are transient ventricular rhythm disturbances, noted first by Lown and coworkers (3) and since by many others (1, 2, 4, 5, 6). Ventricular premature beats and ventricular tachycardia are the most common of these disturbances and occur with greatest frequency in patients on digitalis (7), but ventricular fibrillation (1, 4, 7) and atrioventricular nodal dysrhythmias (1, 4, 6) have been reported. There is some evidence that DC countershock increases both ventricular automaticity and intraventricular conduction time, permitting impulse reentry (6). Application of DC shock to cultured chick myocardial cells induces diastolic depolarization, a property of pacemakers (7).

Electrocardiographic ST-T wave changes have occasionally been associated with DC countershock. In patient studies there have been reports of transient ST segment elevations (1, 4) and depressions(1). There are reports of minor T wave changes (5) as well as T wave inversions persisting over several days (1, 2). In their recent exhaustive analysis of patient reports, Lepeschkin et al. found ST segment elevation usually lasting less than two minutes in 2.9% of 2,341 reported cases and inverted T waves usually lasting several days in

3.5% of 1,520 cases (7). Lown et al. noted that after multiple countershocks, several dogs developed sequential electrocardiographic changes characteristic of myocardial infarction (3).

The possibility of myocardial injury resulting from countershock was suggested by reports of increased serum glutamic oxaloacetic transaminase (SGOT) (1, 2, 4, 5, 8, 9), lactic dehydrogenase (LDH) (2, 8) , and creatine phosphokinase (CPK) (10) in the serum of patients of patients after cardioversion. However, increases in these enzymes were never well correlated with electrocardiographic changes thought to suggest cardiac damage (1, 4, 9). In addition, case reports described patients dying after 140 DC countershocks over 3 days (11) and 34 DC countershocks over 16 hours (12) yet showing no sign of recent myocardial injury at autopsy. Two recent studies have distinguished chemically among isoenzymes of LDH (13) and CPK (14) and have explained post-countershock increases in these enzymes in patients largely on the basis of skeletal muscle release and not myocardial necrosis, although two cardioverted patients did have modest increases in serum MB CPK, the myocardial isoenzyme (14). It has been shown that multiple countershocks can cause pectoral muscle necrosis in man (15), but release of muscle enzymes may occur in the absence of cell death, e.g., in exercise. Skeletal muscle damage after countershock has consequences for the radionuclide assessment of myocardial infarction, as will be described.

The origin of the experimental use of countershock to produce myocardial injury in dogs can be traced to the work of Tedeschi and White, who found that AC and condenser discharge countershock applied directly to the heart surface produced necrosis of underlying epicardium and myocardium (16). Others have carefully characterized the

morphologic changes resulting from directly applied or transthoracic countershock and have shown that the severity of myocardial damage roughly correlates with energy delivered (17, 18). Dahl et al. have demonstrated that the extent and severity of damage after transthoracic countershock in dogs varies inversely with both electrode paddle diameter and time interval between shocks (19, 20). Importantly, it is known that countershock injury spares the myocardial microcirculation. DiCola et al. recently showed in this laboratory that myocardium damaged by transthoracic countershock in dogs has normal regional myocardial blood flow, measured with radioactive microspheres, 24 hours following injury (21). This has profound implications for the use of radionuclide uptake in assessing countershock damage.

Modification of Myocardial Injury by Corticosteroids

Infarct size is thought to be a major determinant of early mortality after myocardial infarction (22). The occurrence of cardiogenic shock is related to the area of myocardial injury (23). Myocardial infarction occurs by a process of evolving cellular events over a period of more than 45 minutes in experimental coronary artery ligation (24) and, perhaps, of several hours in the clinical situation, depending on the availability of collateral blood flow. Attempts have been made both experimentally and clinically to salvage ischemic myocardium (25-27).

One intervention whose value in modifying myocardial necrosis is debated is corticosteroid intervention. Corticosteroids are known to inhibit wound healing, and impaired myocardial infarct healing after corticosteroid administration has been associated with ventricular aneurysm formation (28). However, effects of corticosteroids on mem-

branes, the inflammatory response, hemodynamics and metabolism suggested possible benefit to jeopardized myocardium. In an early study, cortisone given in pharmacologic doses to dogs after coronary artery ligation decreased infarct size and mortality, despite delayed scar formation (29). Other studies did not confirm these observations (30-33).

More recently, Libby et al. used epicardial electrocardiographic ST segment mapping, which correlated with both tissue CPK activity and histologic appearance, to demonstrate significant protection by hydrocortisone of myocardium from infarction if administered within 6 hours of coronary artery occlusion in dogs (34). Spath and coworkers have shown in the cat that methylprednisolone or dexamethasone treatment after coronary occlusion reduce ST segment elevation and limit the rise in plasma CPK activity (35, 36). On the other hand, Osher et al. demonstrated a worsening of ST segment abnormality in methylprednisolone-treated dogs with acute coronary occlusion (37). In addition, Vogel et al. found no difference in myocardial enzyme depletion or nitro-blue tetrazolium dye staining with methylprednisolone administration before or after left circumflex artery occlusion in dogs (38). Kraikitpanitch et al. found no difference in serum SGOT or CPK with hydrocortisone treatment in epinephrine-induced myocardial injury in dogs, but myocardial calcium uptake, considered an indicator of cellular injury, and histologic evidence of necrosis were decreased (39).

Two early, uncontrolled clinical studies suggested that administration of corticosteroid improved survival of patients with severe acute myocardial infarction, especially those in cardiogenic shock (40, 41). There is evidence of a general salutary effect of corticosteroids on the microcirculation in shock (42), which could improve survival in-

dependent of the extent of myocardial damage. A controlled trial in a greater number of patients found that hydrocortisone did not reduce mortality after acute myocardial infarction, even in patients in shock (43). A report on 446 patients treated with hydrocortisone 500 mg a day for 3 days, a dose larger but administered for fewer days than in previous clinical trials, has indicated that the treated group had a lower mortality than did the comparison group of 491 patients (44). However, these data are difficult to evaluate because the control group was cared for by different staffs on different wards, received anticoagulant therapy, unlike the hydrocortisone group, and ambulated earlier.

Using serial serum CPK determinations in patients with acute myocardial infarction in order to predict the ultimate extent of infarction, two tests of corticosteroid therapy in man have produced conflicting results. Morrison et al. found that one or more massive doses of methylprednisolone administered to 66 patients with acute myocardial infarction significantly reduced expected infarct size, predicted by CPK curve-fitting techniques (45). Unfortunately, the two patient groups were not identical, the treated group actually having more severe infarcts, both predicted and completed, than control. In contrast, Roberts et al. gave 12 patients multiple dose methylprednisolone over 48 hours after acute myocardial infarction and found that completed infarcts in the treated group far exceeded what had been predicted, suggesting an exacerbation of myocardial injury by multiple dose corticosteroid. This effect was not seen in 10 patients given single dose methylprednisolone (46).

Several mechanisms have been proposed whereby corticosteroids might favorably alter myocardial injury. These will be reviewed in

the following sections.

Lysosomal Stabilization

Lysosomal membrane stabilization is the most cited mechanism by which corticosteroids might reduce myocardial cellular necrosis (34-36). DeDuve gave the name "lysosome" to cytoplasmic granules rich in hydrolytic enzymes (47). He showed that ischemia causes release of hydrolytic enzymes from rat liver, and commented: "Such a process could conceivably play a causal role in cell death, but it could also occur as a consequence of this phenomenon which may itself be due to more rapid alterations, unrelated to the invasion by lysosomal enzymes." (48). Weissman and others have shown that corticosteroids protect biomembranes, including lysosomal membranes, from a variety of injuries (49-52). Corticosteroids are thought to stabilize membranes directly, perhaps by restricting movement of phospholipid chains, and also to inhibit fusion of lysosomes to the cell membrane (53).

In myocardial ischemia produced by coronary occlusion or asphyxia in dogs, acid phosphatase, β- glucuronidase and other lysosomal enzymes rapidly shift from the particulate or sedimentable to the free or non-sedimentable state (54-56). Myocardial cellular pH falls as lactate accumulates during ischemia (57, 58), approaching the acid pH at which hydrolytic enzymes are maximally active (59). It is thus postulated that conditions are optimal in the ischemic myocardial cell for degradation of essential macromolecules by free lysosomal enzymes, causing cell death. The validity of this postulate has been questioned as a result of studies indicating that ventricular myocardium contains few "classical" or primary lysosomes (60) and that irreversible ischemic

myocardial injury can occur in the absence of electron microscopic evidence of lysosomal membrane disruption (61). These results suggested that perhaps acid hydrolases recovered by ultrafiltration in the non-sedimentable cytoplasmic fraction are released not by cardiac lysosomes but by endothelial and interstitial cell organelles. However, in vitro human glial lysosomes have been made to leak acid phosphatase through membranes appearing ultrastructurally intact (62). In addition, Hoffstein et al. have demonstrated in the myocardial cell that acid hydrolases are intimately associated with the sarcoplasmic reticulum, and that two hours after coronary occlusion in dogs, there is decreased membrane-bound enzyme activity in the endocardium (63, 64).

That corticosteroids improve myocardial lysosomal resistance to ischemia is indicated by the studies of Spath and coworkers, who found that administration of methylprednisolone or dexamethasone before or after coronary ligation in the cat prevented or reduced the decline in acid hydrolases in ischemic zones and the shift from bound to free state (35, 36). Busuttil et al. found less β-glucuronidase in coronary sinus blood of dogs with ischemic cardiac arrest treated with methylprednisolone than control (65). Hoffstein et al. also showed that methylprednisolone permitted the retention of hydrolytic enzymes by endocardial sarcolemma and prevented the redistrubution of enzymes to the non-sedimentable state in dogs with coronary occlusion (64). Finally, Replogle et al. noted that 10 patients given intravenous dexamethasone before and after cardiopulmonary bypass had lower serum β-glucuronidase at 4 hours and a "better general appearance" postoperatively than control (66), but it may be unfair to infer that myocardial damage was avoided.

Two recent reviews express skepticism that corticosteroids could

have as their primary action in vivo the stabilization of lysosomal membranes (67, 68). Both articles point out that tissue corticosteroid concentration would have to approach 10^{-4}M, a level higher than it seems possible to achieve therapeutically. They also point to the inconsistent correlation of steroid chemical structure with biological activity. For example, cortisone used in vitro is as effective as cortisol, but only cortisol is effective in vivo. Lastly, both agree with deDuve (48) that release of lysosomal enzymes may not be the cause of cell death at all.

Okuda et al. have recently found that the isolated, perfused rat heart accumulates tritiated-methylprednisolone and -dexamethasone largely in the plasma membrane cell fraction, and that during acute ischemia, activity of 5'-nucleotidase, a plasma membrane marker, is preserved in corticosteroid-treated hearts compared with control (69). This supports the belief that corticosteroids interact with cell membranes, but it does not exclude other effects, and it may not apply in vivo.

Anti-inflammatory Effects

Glucocorticoids are known to alter many aspects of immune and inflammatory responsiveness. They are lympholytic, inhibit leukocyte adherence to vascular walls, and reduce platelet aggregation, diapedesis and cellular infiltration (68). Systemic corticosteroids are vasoconstrictive on both normal and injured capillary beds, and vasoconstriction could decrease extravasation of cells and fluid (68). The inflammatory reaction which accompanies myocardial injury, whether ischemic or countershock-produced, may exacerbate tissue injury. In addition to reducing the inflammatory infiltrate, corticosteroids could stabilize inflammatory cell phagocytic vacuoles and thus reduce heterolytic activity.

Increased Coronary Blood Flow

Johnson et al. first demonstrated that after chronic administration of cortisone to dogs after coronary occlusion, their hearts had more prominent vascularity than control animals, determined by x-ray after injection of contrast (29). This apparent improvement in collateral blood supply was thought to explain their improved survival and smaller infarcts. Eckstein was unable to replicate these results, finding no difference in vascular pattern or in interarterial anastomoses as estimated by clamping coronary inflow and recording perfusion pressure distally (33). Recently, several groups have confirmed the original observation that corticosteroid administration increases coronary blood flow (70-74). Improvement in coronary blood flow of treated dogs has been shown variously to occur in normal (non-ischemic) myocardium (70), in ischemic myocardium (71, 72), in both normal and ischemic myocardium (74), and in ischemic but not in normal myocardium (73). It has been theorized that corticosteroid administration might enhance the "ischemic stimulus" to opening of collateral vessels (73). It should be noted here that since countershock myocardial injury is non-ischemic, it seems unlikely that an increase in coronary blood flow would modify necrosis.

Hemodynamic Effects

A number of studies have indicated that corticosteroids have a positive inotropic effect, increasing- or averting a decrease in -the maximal rate of rise of left ventricular pressure (LV dP/dt) (65, 70-72, 74-76). Other investigators have been unable to demonstrate an increase in LV dP/dt after corticosteroid traetment in acute ischemia (37, 73). Da Luz et al. recently showed that dogs treated with methylprednisolone after coronary occlusion had greater systolic shortening of the

ischemic segment, indicating better regional myocardial function, than control after three hours of ischemia, and also after one hour reperfusion (77). Tecklenberg et al. found that the transient positive inotropic response to corticosteroids was augmented by cardiac denervation in the dog, and reduced by β-adrenergic blockade with propranolol or by acute adrenalectomy; they suggest that corticosteroids release and/or potentiate endogenous catecholamines, which then mediate the positive inotropic effect (76).

Another line of evidence indicates that the inotropic state of rat myocardium is regulated by the cyclic nucleotides, cAMP and cGMP, and that increases in myocardial cGMP are associated with reduced contractility (78). Infarcted canine myocardium contains reduced levels of cAMP and increased cGMP (79). Adrenalectomy increases cGMP in rat skeletal muscle, an effect reversed by dexamethasone (80). Butsuttil et al. found that methylprednisolone pretreatment reduced the elevation of left ventricular myocardial cGMP produced by ischemic cardiac arrest in dogs; this was associated with a preservation of LV dP/dt (65).

A positive inotropic effect of corticosteroids could potentially provide either help or hindrance to ischemic myocardium. Inasmuch as increases in the contractile state of the myocardium increase oxygen demand, hypoxic tissue would face further jeopardy. Maroko et al. have shown that other interventions with positive inotropic effects increase the extent of ischemic injury after coronary occlusion in dogs (25, 81). However, in the event of pump failure, an inotropic stimulus might be life-saving.

Other hemodynamic determinants of myocardial oxygen demand may be influenced by corticosteroids. Left ventricular end diastolic pressure,

or preload, is a determinant of intramyocardial tension, and has been found both to decrease (70, 71, 76) and not to change significantly (37, 74). Arterial or left ventricular pressure has been shown to fall transiently soon after corticosteroid administration (34, 37,42, 71, 73, 82), perhaps because of a direct vascular effect with fall in systemic vascular resistance (76), and one group has invoked this as a potential means of myocardial salvage. Heart rate after corticosteroid administration has increased (37, 83), decreased (71), or remained unchanged (34, 73, 77). Cardiac index has increased (42, 71), or changed insignificantly (84). Cardiac output has not changed, either in dogs with closed chest coronary occlusions (37), or in patients with septic shock (85).

Metabolic Effects

The metabolic consequences of acute myocardial ischemia have been well characterized (86, 87). Aerobic metabolism requires oxygen, and there is evidence that corticosteroid administration increases oxygen delivery to and uptake by normal (70) and ischemic (71, 73) myocardium. Glucocorticoid may additionally decrease myocardial oxygen consumption (88) and shift the oxyhemoglobin dissociation curve to the right, promoting oxygen release (89). Busuttil et al. found that dogs treated with methylprednisolone had a greater arterial pO_2 than control during recovery from ischemic cardiac arrest (65), and Motsay et al. found that corticosteroid-treated dogs with coronary occlusions and patients in cardiogenic shock consumed more oxygen than those untreated, perhaps because of a non-specific improvement in viscerocutaneous perfusion (42).

Reduced oxygen supply to the myocardium causes shift in cellular oxidation-reduction state so that the reduced form of nicotinamide adenine dinucleotide (NADH) predominates over the oxidized form (NAD). Shifts in cytoplasmic NAD:NADH ratios are grossly reflected by shifts in intracellular lactate:pyruvate ratios, indicating shifts from aerobic to anaerobic metabolism. Myocardial ischemia has been expressed in terms of lactate extraction and production, but many variables modify such measurements, including the requirement for "washout" of intracellular lactate into the venous drainage which is sampled (90). Brachfeld and Da Luz et al. (77) have both demonstrated improved lactate balance in ischemic myocardium with corticosteroid treatment, suggesting reduced anaerobic metabolism and increased tissue viability. On the other hand, others have been unable to show a significant difference in lactate balance (37, 65). Yet others have found increased lactate in coronary sinus blood with corticosteroid treatment in myocardial ischemia, and explain this as a consequence of improved coronary blood flow and enhanced "washout" (73, 84).

Masters et al. have shown that ischemic myocardium has increased uptake of glucose, and decreased uptake of pyruvate, lactate and free fatty acids, reflecting transport gradients resulting from anaerobic metabolism. Methylprednisolone increased free fatty acid and lactate uptake, and markedly increased pyruvate production (73). Increased production of pyruvate, an essential substrate for anaerobic metabolism, may be due to the known enhancement of gluconeogenesis by glucocorticoid. Corticosteroids have also been shown to increase serum glucose in man (83) and dog (76) and to accelerate glucose turnover in the dog (92).

Myocardial potassium balance has been used as an indicator of

cellular integrity during ischemia, and there is evidence both for improvement (65, 77) and for no change (37) as a result of corticosteroid administration.

Assessment of Myocardial Injury by ^{99m}Tc-PYP

Technetium-99m stannous pyrophosphate (^{99m}Tc-PYP) is a well known bone scanning agent which has recently been established by Willerson and colleagues as a clinical tool useful in the detection of acute myocardial infarction in man (93, 94) and as a research tool permitting evaluation of experimental infarction in the dog (95). ^{99m}Tc-PYP myocardial scintigrams become positive 12 to 16 hours after coronary artery ligation or clinical onset of infarction, increase in intensity over the next 12 to 36 hours, remain positive up to 6 days after the onset of infarction and then fade, usually becoming negative by the 14th day after coronary occlusion in dogs and within 7 days after the onset of infarction in patients (93-95). The mechanism by which ^{99m}Tc-PYP accumulates in infarcted myocardium is believed by Buja et al. to involve binding to abnormal calcium deposits in the mitochondria of dead cells (96, 97). Mitochondrial calcium uptake accompanies irreversible cellular injury, and is postulated to be a common pathogenic mechanism for muscle necrosis (98). Fleckenstein hypothesizes that calcium uptake impairs cellular phosphorylating capacity and adenosine triphosphate (ATP) synthesis, resulting in cell death (99). In dogs with experimental infarcts, ^{99m}Tc-PYP myocardial uptake has been noted to occur at about the same time (at 1-2 days, disappearing between 7 and 13 days) and in approximately the same location (in "peripheral zones" of infarction) as myofibrillar calcification, ultrastructurally resembling hydroxy-

apatite crystals within mitochondria (96). Subcellular distribution studies by Dewanjee and Kahn in the rabbit indicate that ^{45}Ca and ^{99m}Tc-PYP taken up by infarcted tissue are associated mainly with the soluble protein fraction, not the mitochondrial fraction; they postulate that ^{99m}Tc-PYP binds with denatured cellular macromolecules to form polynuclear complexes- independent of cellular calcium uptake (100). Incubation of fetal mouse hearts in calcium-free culture medium does not reduce ^{99m}Tc-PYP uptake after metabolic injury (101).

Attempts have been made to use ^{99m}Tc-PYP scanning and tissue assay to quantify infarct size in clinical and experimental situations. The size of experimental anterior wall infarcts has been estimated with some success from the scintigraphic ^{99m}Tc-PYP "hot spot" area, which correlates with infarct weight (102, 103). Difficulties have been encountered in attempts to evaluate the extent of infarction in terms of the intensity, or count density, of the radionuclide scan. Positive myocardial scans after proximal left anterior descending coronary artery occlusion in dogs typically show "doughnut" patterns, with marked peripheral concentration of radioactivity surrounding central zones of much lower activity (96, 104). Studies using experimental infarcts in dogs have shown by direct tissue assay that myocardial uptake of ^{99m}Tc-PYP does not correlate well with either tissue CPK depletion (105) or the histologic extent of necrosis (104, 106). The reason for this has been established by correlation of ^{99m}Tc-PYP myocardial uptake with regional myocardial blood flow, estimated by tissue distribution of radioactive microspheres (105-108). Zaret et al. found that in experimental infarction, ^{99m}Tc-PYP is taken up maximally by tissue with 30-40% of normal blood flow, and progressively less with greater degrees of ischemia (105). This indicates

that accumulation of tracer in necrotic myocardium requires delivery to the site of necrosis. The characteristic "doughnut" scan image results from blood flow in the center of the infarct inadequate to permit delivery of tracer. Thus, ^{99m}Tc-PYP distributes unevenly in myocardial infarcts. This limits quantification of the extent of necrosis to measuring the area of the scintiscan "hot spot" (102, 103).

An additional problem is that the scan image is a two dimensional representation of a three dimensional object (109, 110); thus, activity in tissues above and below the area of interest are superimposed, reducing contrast, and gamma radiation is attenuated as it passes through tissue between the area of interest and the detector. This problem is only in part alleviated by scanning in several positions. Another apparent limitation to the quantitative use of ^{99m}Tc-PYP scanning is its lack of complete specificity. Some patients with unstable angina pectoris and, occasionally, patients with simple angina pectoris have been found to have diffusely positive ^{99m}Tc-PYP scans, apparently unrelated to the severity of their coronary artery disease, determined angiographically (111-113). It has been suggested that tracer may sequester in ischemic myocardium, but the presence of necrotic foci amidst otherwise reversibly injured tissue has not been ruled out.

Positive myocardial scans have been reported in patients with left ventricular aneurysm (114) and in dogs after experimental cardiac contusion (115). Pugh et al. have shown that there is abnormal uptake of ^{99m}Tc-PYP in pectoral muscle of dogs 24 hours after transthoracic countershock (116). After cardioversion, several patients have reportedly shown sites of ^{99m}Tc-PYP accumulation on scans not corresponding to electrographic localization of myocardial infarction; it is felt that

these "false positive" myocardial scans represent radionuclide uptake in damaged skeletal muscle (117).

DiCola et al. have shown in the dog that myocardium damaged by high energy transthoracic countershock accumulates ^{99m}Tc-PYP in amounts far greater than ischemic infarcts (21). Abnormal radionuclide uptake was subepicardial to full thickness, corresponding to the depth of tissue necrosis, demonstrated histologically and histochemically. At the same time, regional myocardial blood flow, measured with radioactive microspheres 24 hours following countershock, was maintained (21). Because countershock injury is non-ischemic, myocardial uptake of ^{99m}Tc-PYP should correlate directly with the extent of necrosis, and permit quantitative evaluation of myocardial damage. This provided impetus for the experiments to be described.

METHODS

Effects of Methylprednisolone on Countershock Injury

Twenty-five mongrel dogs of either sex ranging in weight from 12.3 to 19.5 kg were anesthetized with intravenous sodium pentobarbital, 30 mg/kg, intubated and placed on a Harvard respirator. Twelve of the dogs were given methylprednisolone sodium succinate (Solu-Medrol, Upjohn), 30 mg/kg, as an IV bolus, and a weight-matched group of 13 dogs received an equivalent volume of its vehicle. Ninety minutes later, with each dog lying in the left anterior oblique position, 4 cm diameter paddle electrodes were applied with electrode paste (Redux Paste, Hewlett Packard) at the points of maximal cardiac impulse on the shaved left and right chest, and two 400 watt-second electroshocks (320 watt-seconds delivered across a 50 ohm resistance) separated by one minute were administered via a B-D Electrodyne Model ELD-5B DC pulse external defibrillator. Animals were medicated with lidocaine 100 mg IV two minutes before shock and as necessary afterward to eliminate ventricular ectopy. Electrocardiographic recordings were made before countershock, and 5 minutes and 60 minutes after, with a precordial electrode placed successively at 15 points mapped out in advance in three rows of 5 points in the left 4th, 5th and 6th intercostal spaces, from sternum to to left mid-axillary line.

Twenty-four hours following countershock, dogs were again anesthetized, then injected with approximately 10 millicuries of ^{99m}Tc-stannous-pyrophosphate (Mallinckrodt). Each dose was measured precisely in a RadX dose calibrator. One hour later, animals were sacrificed with IV potassium chloride, and hearts were removed, trimmed of epicardial fat, and cut into approximately 25 1-2 gm transmural samples, including

all grossly abnormal tissue with wide border zones, and 5 normal samples from the posterior left ventricle. Samples were divided into epicardial and endocardial halves, weighed and counted for ^{99m}Tc activity in a Picker Spectroscaler III A well-type scintillation counter with a 3" x 3" sodium iodide crystal at a window of 100-140 keV. Sample radioactivity was calculated as counts per minute per gram of tissue per millicurie injected, and was correctedfor decay of ^{99m}Tc (half-life = 6.0 hours) from the exact time of sacrifice of the animal.

Before dissection, 7 hearts from each group were quantitatively imaged using a computerized multicrystal scintillation camera (Baird-Atomic System 77). Great vessels and atria were removed, and ventricles were scanned for 16 minutes with the anterior epicardial surface 5 cm from the 2" high-resolution, parallel hole collimator. Data were stored on magnetic tape after uniformity flood and environmental background correction. Cardiac scan images consistently demonstrated two zones of increased ^{99m}Tc-PYP uptake, one in the area of the right ventricle and one near the apex of the left ventricle, as well as low background cardiac activity (Figure 1a.). Computerized data processing of each abnormal scan involved, first, increasing the minimal count level displayed as non-zero data to the extent judged necessary for elimination of background activity. Each abnormal zone was then divided by itself to obtain a unity image (Figure 1b.), defining the "hot spot" in terms of numbers of detector crystals, each 7.73 mm^2. The original, background-corrected scan was then divided by the unity image, giving the number of counts in the abnormal zone. This permitted expression of abnormal zone activity in terms of accumulated $counts/cm^2$.

In 5 hearts from control and 4 from methylprednisolone-treated (MP) dogs, 40-80 mg full-thickness portions of each tissue sample were assayed

for creatine phosphokinase (CPK). These samples were immediately frozen in liquid nitrogen, homogenized in 2 ml of 0.01 M phosphate buffer (pH 7.2) containing 0.01 M sodium EDTA, 0.01 Tris HCl and 0.01 M glutathione, and centrifuged at 10,000 RPM for ten minutes. One ml of the supernatant was used for measurement of CPK by the method of Rosalki (118).

Transmural biopsies were taken for histopathologic study from the centers of abnormal zones in 8 control and 8 MP dogs. These were fixed in 10% formalin, embedded in paraffin, cut, and stained with hematoxylin and eosin, Masson trichrome and hematoxylin basic fuchsin picric acid stains (119). Sections were examined for evidence of necrosis, hemorrhage, edema and cellular reaction, and for depth of myocardial injury.

Thirteen biopsies from each group were graded semiquantitatively as: 0, no definite necrosis; 1+, scattered, mild, but definite necrosis; 2+, subepicardial necrosis (limited to outer 1/2 of myocardium) with moderate to severe tissue reaction; 3+, transmural necrosis (greater than 1/2 full-thickness) with moderate to severe tissue reaction.

Effects of Methylprednisolone on ^{99m}Tc-PYP Distribution and Excretion

The effects of methylprednisolone on the distribution in blood and bone and on the clearance and excretion of ^{99m}Tc-PYP were studied by the following protocol. Thirty-four dogs were divided into 17 weight matched pairs. One dog of each pair received methylprednisolone, 30 mg/kg IV, and the other its vehicle 24 hours prior to injection of about 10 millicuries ^{99m}Tc-PYP. Animals were always studied simultaneously in pairs. In 22 dogs (11 pairs), the urinary bladder was

catheterized and emptied by external massage just prior to injection of ^{99m}Tc-PYP. The bladder was emptied at 10 minute intervals thereafter until sacrifice, at which time the abdomen was entered and the bladder emptied of residual urine. The volume of each sample was recorded, and a 2 ml aliquot was saved for well-counting. Two ml samples of arterial blood were withdrawn through a femoral artery catheter before PYP injection, and at 1, 2, 5, 10, 20, 30, 40, 50 and 60 minutes after injection, and saved for well-counting. Twelve dogs (6 pairs) studied earlier had fewer arterial blood samples and no urine collected, a point clarified in Tables 4 and 6. Animals were sacrificed with IV potassium chloride at 60 minutes.

Plasma was separated by centrifugation from arterial blood drawn at the time of sacrifice from 5 pairs of dogs. Separation of plasma protein was then accomplished by a modification of the method of Somogyi (134). 0.5 ml plasma, 3.5 ml H_2O and 0.5 ml zinc sulfate reagent (100 gm $ZnSO_4 \cdot 7H_2O$ and 40 ml 6.25N H_2SO_4 diluted to 1000 ml with H_2O) were mixed, and 0.5 ml of 0.75N sodium hydroxide was added dropwise. The solution was stirred for 20 minutes and centrifuged 10 minutes, the supernatant was discarded, the precipitate was resuspended in distilled H_2O and recentrifuged, and the precipitate was saved for ^{99m}Tc well-counting.

In 6 pairs of dogs, three 2-3 gm cortical bone samples were taken from the right and left 4th ribs, posterior iliac crests and femoral heads. As in the countershock studies, 5 1-2 gm normal left ventricular myocardial samples were taken from all dogs studied. All tissue samples were weighed and counted for ^{99m}Tc activity. At the same time a 0.1 ml aliquot from the same batch of ^{99m}Tc-PYP as the injected dose was diluted 1:1000 and counted each experimental day to permit calibration of

the well-counter for CPM per mCi. This permitted expression of ^{99m}Tc-PYP kinetic data in terms of % injected dose per gram of tissue, or per volume of excreted urine.

Ninety minutes before radionuclide injection in 6 pairs of dogs, an intravenous infusion of 5% dextrose in water (D5W) was begun at a rate of 300 ml per hour and continued throughout the experiment. In 5 pairs, to be assured of adequate urine output, 450 cc D5W per hour were infused beginning 90 minutes before PYP injection; also, to facilitate measurement of creatinine clearance, creatinine, 20 mg/kg in 20 cc D5W, was administered as an IV bolus, and then infused at 6 mg per minute in the D5W drip. One ml aliquots of urine collected at 10 minute intervals and of plasma separated by centrifugation from arterial blood drawn at 20 minute intervals were frozen and saved for creatinine determination utilizing a Technicon autoanalyzer. Plasma sodium concentration was measured in these 5 pairs of animals before PYP injection and at the time of sacrifice (60 minutes) with the Instrumentation Laboratory Flame Photometer 143.

RESULTS

Validation of ^{99m}Tc-PYP Assessment of Countershock Injury

The validity of utilizing myocardial ^{99m}Tc-PYP uptake as an index of myocardial necrosis in the countershock model was examined by comparison with an independent index of necrosis, tissue creatine phosphokinase (CPK) depletion. Fifty transmural samples from grossly abnormal as well as apparently normal border zones in three control animals were analyzed. PYP and CPK activity were expressed in the usual way (104-108) as an activity ratio between each myocardial sample and the mean of 5 normal samples from the posterior left ventricle - the sample:normal ratio (S:N ratio). In the non-ischemic countershock model, as shown in Figure 2, PYP S:N ratios, plotted on the ordinate, correlated inversely with tissue CPK S:N ratios, on the abscissa, with r = -0.83. In other words, tissue PYP uptake 24 hours following countershock correlates well with tissue CPK depletion.

Effects of MP on Countershock Injury: ^{99m}Tc-PYP Assessment (Table 1)

The 13 control and 12 methylprednisolone-treated (MP) dogs did not differ significantly in weight. For each animal, an average PYP sample:normal ratio was obtained by computing the average of its 5 transmural myocardial samples with the greatest abnormality in S:N ratio. The MP group had a significantly greater mean average PYP S:N ratio than control, 41.4 ± 6.5 vs. 27.0 ± 2.2, mean ± SEM, $p < 0.05$ by group t test. This difference is demonstrated graphically in Figure 3, left panel, and might lead one to conclude that the methylprednisolone-treated group had greater necrosis than control. However, when one looks at absolute

TABLE 1 ^{99m}Tc-PYP Assessment of Countershock Injury

Animal	Weight (kg)	Sample:Nl Ratio	Abnormal Zone (CPM/g/mCi x 10^{-3})	Normal Zone	Abnormal Zone Weight (g)	Abnormal Image Activity (counts/cm^2 x 10^{-3})
CONTROL						
a	19.5	26.5	205.8	7.8	14.8	
b	18.6	27.3	362.4	13.2	14.7	
c	19.1	25.7	266.1	10.3	15.0	
d	16.8	24.4	578.5	23.7	10.8	
e	18.2	44.5	519.5	11.7	16.7	
f	16.8	20.4	206.4	10.2	12.3	
g	18.0	33.0	110.0	3.4	7.0	6.78
h	16.8	32.5	91.3	2.8	8.3	7.96
i	18.2	16.1	65.7	4.1	3.9	4.09
j	15.9	22.9	102.8	4.5	9.3	3.30
k	17.3	14.9	151.1	10.1	6.1	2.65
l	17.3	28.5	118.8	4.1	4.1	5.76
m	15.5	33.7	211.9	6.3	13.3	5.76
Mean	17.5	27.0	230.0	8.6	10.4	5.19
SEM	0.3	2.2	45.4	1.6	1.2	0.73
METHYLPREDNISOLONE						
A	18.6	13.5	126.6	9.3	12.6	
B	17.5	26.9	216.5	8.0	8.4	
C	17.7	38.0	241.1	6.4	20.6	
D	17.3	12.2	58.6	4.8	3.7	
E	17.3	38.3	296.8	7.7	10.2	
F	18.2	70.1	84.5	1.2	10.3	5.17
G	17.3	40.2	43.6	1.1	11.3	3.16
H	17.7	65.4	88.2	1.4	10.8	5.32
I	18.2	77.9	93.9	1.3	7.5	6.04
J	15.5	29.2	70.9	2.4	9.2	2.78
K	12.3	25.2	106.4	4.3	9.4	3.31
L	13.6	60.4	346.1	5.7	9.7	8.60
Mean	16.8	41.4	147.8	4.5	10.3	4.91
SEM	0.6	6.5	29.2	0.9	1.1	0.78
	NS	$p < 0.05$	NS	$p < 0.025$	NS	NS

abnormal zone activity, expressed in CPM/g/mCi, again by using the average of each animal's 5 most radioactive samples, one finds no significant difference and, in fact, the control group's mean is higher (Figure 3, center). Normal zone absolute PYP activity was, on the other hand, significantly less in the MP group, $p<0.025$ (Figure 3, right). Thus, the increased abnormality of the sample:normal ratio in MP dogs appears to be a function of reduced normal zone activity, rather than expected increase in abnormal zone activity. Further data corroborating this interpretation will be presented.

Abnormal myocardial ^{99m}Tc-PYP uptake was consistently transmural, though more epicardial than endocardial in distribution. An estimate of abnormal zone weight was made by summing the weights of all epicardial and endocardial tissue samples with abnormal ^{99m}Tc-PYP accumulation, defined as an S:N ratio greater than 3:1. Mean abnormal zone weights derived in this way were almost identical, with control, 10.4 ± 1.2 grams, vs. MP, 10.3 ± 1.1 grams (Table 1, Figure 4).

Cardiac scan images consistently demonstrated two zones of rather uniformly abnormal radionuclide uptake, one in the area of the right ventricle and one near the left ventricular apex (Figure 1a.). PYP count densities derived by computerized data processing were equivalent in the two groups (Table 1, Figure 4).

Effects of MP on Countershock Injury: CPK Assessment (Table 2)

The 5 control and 4 methylprednisolone-treated animals for which CPK data were obtained did not differ significantly in weight. For each dog, an average CPK sample:normal ratio was calculated as for PYP by computing the average of its 5 tissue samples with the greatest abnormality in S:N ratio. (In this case, abnormality implies a fall

TABLE 2 CPK Assessment of Countershock Injury

Animal		Weight (kg)	Sample:Nl Ratio	Abnormal Zone (milliIU/mg)	Normal Zone (milliIU/mg)
CONTROL					
a		19.5	0.13	152	1132
b		18.6	0.12	146	1196
c		19.1	0.42	523	1250
d		16.8	0.27	109	393
e		18.2	0.11	127	1179
	Mean	18.4	0.21	211	1030
	SEM	0.5	0.06	78	160
METHYLPREDNISOLONE					
A		18.6	0.16	213	1330
B		17.5	0.19	321	1665
C		17.7	0.24	254	1058
D		17.3	0.38	103	270
	Mean	17.8	0.24	223	1080
	SEM	0.3	0.05	46	297
		NS	NS	NS	NS

in S:N ratio, indicating CPK depletion.) Figure 5 shows graphically that there were no differences between the two groups in mean CPK S:N ratio, which was .21 ± .06 in the control group vs .24 ± .05 in the MP group, mean ± SEM; nor were there differences in absolute tissue CPK, expressed in mIU/mg, in abnormal zones or normal zones.

Effects of MP on Countershock Injury: Precordial ST Segment Mapping

ST segment amplitude was measured in all recordings at 0.08 seconds after the onset of the QRS complex with the PR segment as baseline (19). The sum of all ST amplitudes exceeding 2 mm (ΣST) and the number of lead positions with such ST elevation (NST) were calculated. There were no significant differences between the two groups in either ΣST or NST before countershock (time 0) or at 5 minutes or 60 minutes afterward (Figure 6). There were several animals in both groups which had slight or absent ST segment elevation, yet substantial myocardial necrosis, demonstrated histologically and by ^{99m}Tc-PYP uptake. This accounts for relatively large standard errors. ST data are included for one control animal for which PYP data are not available because the animal died several hours after countershock.

Effects of MP on Countershock Injury: Histopathologic Assessment

On gross inspection, zones of injury were apparent as pale yellow discoloration of epicardium and underlying myocardium, often extending transmurally to the endocardial surface. There were consistently two distinct, circular areas of right ventricular and left ventricular damage, corresponding to "entrance" and "exit" wounds. All abnormal zones studied histologically and histochemically showed qualitatively similar transmural disruption of muscle fibers, coagulation necrosis, interstitial edema, hemorrhage and mixed cellular infiltration, with mononuclear leukocytes predominating over polymorphs (Figure 7). There was no clear difference in mean histopathologic grade between the two groups (Figure 7).

Effects of MP on ^{99m}Tc-PYP Distribution

Arterial blood ^{99m}Tc-PYP activity is presented in Table 3 for control and methylprednisolone-treated animals at all times sampled, and clearance curves derived from these data are plotted in Figure 8. There is no statistically significant difference (group t test) in mean blood PYP activity between the two groups at any time shown. Yet, at each time between 10 and 60 minutes after injection, blood PYP activity in the MP group is slightly less than control. That this finding is not more impressive may be a result of another apparent effect of MP treatment, a dramatic increase in urine volume, 282.1 ± 76.0 (MP) vs. 62.8 ±24.4 (control) milliliters in one hour, $p<0.005$ (Table 7). A large water load was given to all but 6 pairs of animals to facilitate measurement of creatinine and PYP clearance, and the increased urine output may reflect improved ability to excrete a water load in the MP group, as

TABLE 3 ^{99m}Tc-PYP Blood Clearance in CPM/ml/mCi x 10^{-4}

	1	2	5	10	20	30	40	50	60	minutes
CONTROL										
1									4.3*	
2									2.0*	
3									3.4*	
4					8.8		6.1		4.0*	
5				16.0	11.9	9.7	8.3	7.3	6.6*	
6				12.0	7.8	5.5	5.2	4.8	3.5*	
7	22.4	18.7	12.4	8.9	5.9	4.7	3.7	3.1	2.9*	
8	49.0	34.1	19.9	13.7	9.1	7.1	4.8	4.5	3.8	
9	24.8	22.5	13.8	8.2	5.3	3.8	3.1	2.5	2.1	
10	25.6	16.6	10.2	7.2	4.5	3.1	2.7	2.3	2.0*	
11	18.9	12.1	7.4	4.9	3.1	2.3	1.9	1.6	1.4*	
12	16.9	11.1	6.5	4.4	2.9	2.1	1.6	1.4	1.2	
13	24.4	17.8	11.2	7.9	5.3	4.3	3.3	2.7	2.5	
14	22.3	15.9	10.0	7.1	4.8	3.6	2.8	2.3	1.9	
15	22.0	16.0	11.4	7.0	4.7	3.5	2.8	2.3	2.0	
16	18.5	13.9	9.1	6.2	4.1	3.1	2.5	2.1	1.8	
17	22.6	15.9	9.7	6.8	4.6	3.5	2.9	2.4	2.1	
Mean	24.3	17.7	11.1	8.5	5.9	4.3	3.7	3.0	2.8	3.3**
SEM	2.6	1.9	1.1	0.9	0.7	0.6	0.5	0.4	0.3	0.5
%Dose/L	38.5	28.1	17.6	13.5	9.4	6.8	5.9	4.8	4.4	5.2
%Dose/Tot Blood Vol	55.6	40.5	25.3	19.4	13.5	9.8	8.5	6.9	6.3	7.5
METHYLPREDNISOLONE										
1									1.4*	
2									1.5*	
3									2.6*	
4					7.8		4.8		3.8*	
5				7.1	4.3	5.3	4.7	3.7	2.8*	
6				6.5	4.1	3.3	2.3	1.8	1.5*	
7	25.0	18.2	12.0	7.5	4.6	3.0	2.5	1.9	1.9*	
8	33.4	25.6	18.1	11.9	9.0	6.8	5.0	4.1	3.4	
9	38.9	22.6	11.6	9.1	5.1	3.8	3.1	2.4	2.3	
10	21.8	14.3	9.2	6.9	4.6	3.6	2.8	2.3	2.0*	
11	23.1	15.0	9.0	6.0	3.5	2.6	2.0	1.8	1.4*	
12	19.2	13.4	8.7	6.0	3.7	2.7	2.1	1.7	1.3	
13	25.6	18.0	11.6	7.8	4.9	3.5	2.9	2.4	2.1	
14	23.9	18.6	12.6	9.7	7.1	6.0	5.6	4.9	4.5	
15	15.6	11.6	7.9	5.4	3.2	2.2	1.8	1.3	1.2	
16	17.7	13.0	8.5	6.2	4.2	3.4	2.9	2.4	2.2	
17	17.6	13.1	8.1	5.5	3.3	2.3	1.9	1.5	1.3	
Mean	23.8	16.7	10.7	7.4	5.0	3.7	3.2	2.5	2.2	2.1**
SEM	2.1	1.3	0.9	0.5	0.4	0.3	0.3	0.3	0.2	0.3
	NS	NS	NS	NS	NS	NS	NS	NS	NS	
%Dose/L	37.7	26.5	17.0	11.7	7.9	5.9	5.1	4.4	3.5	3.3
%Dose/Tot Blood Vol	54.7	38.4	24.6	17.0	11.5	8.6	7.4	6.4	5.1	4.8

* 60 minute ^{99m}Tc-PYP activities of pairs of animals not water-loaded, or with similar urine output.

** Means for these 60 minute activities, $p < 0.025$.

will be discussed. In theory, the MP dogs had less expansion of their vascular volume by the administered water load. This would have raised the concentration of all circulating substances, including PYP, relative to the control group, potentially masking a blood PYP-lowering effect of methylprednisolone. Supporting this is the fact that measurement of sodium concentration by flame photometry in plasma drawn from 5 pairs of water-loaded animals before PYP injection (time 0) and at the time of sacrifice (60 minutes) revealed a significantly smaller drop in plasma sodium in the MP group, $p<0.025$, whether expressed as mean absolute change or mean fractional change in sodium concentration (Table 4). In addition, if one considers blood PYP activity only in pairs of animals not water loaded (control and MP, dogs 1-6) or, if water loaded, with similar urine output (control and MP dogs 7, 10 and 11), as noted by asterisks in Table 3, one finds a statistically significant reduction in blood PYP, $p<0.025$, in the methylprednisolone-treated group at 60 minutes after PYP injection.

Data are presented in Table 5 giving distribution of PYP 60 minutes after injection in whole blood, plasma, bone, and myocardium. Mean dog weights were the same in the two groups. Whole blood and plasma PYP activity are not statistically different, presumably for the reasons just cited. Plasma protein-binding, measured in 5 pairs of animals, did not differ between the two groups, nor was there a difference in bone PYP uptake, determined in 6 pairs of animals. Myocardial PYP uptake was less in the methylprednisolone-treated group than control, as noted earlier in countershock animals, but fell short of statistical significance.

TABLE 4 Plasma Sodium in Water-Loaded Animals

		[Na] (mEq/L) 0 min	60 min	Δ [Na]	$\frac{\Delta [Na]}{[Na]_{0\ min}}$
CONTROL					
13		130.2	128.2	2.0	0.015
14		139.3	129.8	9.5	0.068
15		132.8	126.3	6.5	0.049
16		138.0	133.9	4.1	0.030
17		130.0	123.6	6.4	0.049
	Mean	134.1	128.4	5.7	0.042
	SEM	1.9	1.7	1.3	0.009
METHYLPREDNISOLONE					
13		127.8	124.8	3.0	0.023
14		130.8	129.0	1.8	0.014
15		136.5	134.3	2.2	0.016
16		136.0	132.6	3.4	0.025
17		133.1	130.4	2.7	0.020
	Mean	132.8	130.2	2.6	0.020
	SEM	1.6	1.6	0.3	0.002
		NS	NS	$p<0.025$	$p<0.025$

TABLE 5 Distribution of ^{99m}Tc-PYP 60 Minutes Following Injection

Animal	Weight	Whole Blood	Plasma	Plasma Protein Bound	Bone	Myocardium
	(kg)	(CPM/ml/mCi x 10^{-4})			(% dose/g)	(CPM/g/mCi x 10^{-3})
CONTROL						
1	18.2	3.98				11.7
2	16.8	6.57				23.7
3	19.1	3.48				10.3
4	13.6	4.28				12.3
5	16.8	1.95				4.8
6	19.5	3.36				7.9
7	23.2	2.93	4.57		0.023	7.7
8	10.0	3.78	5.55		0.033	10.7
9	19.1	2.08	2.77		0.014	13.4
10	24.5	2.01	3.21		0.013	5.1
11	25.5	1.44	2.28		0.007	3.6
12	21.4	1.23	1.68		0.019	3.6
13	16.4	2.57	3.45	3.02		6.8
14	18.2	1.93	2.83	2.26		5.1
15	24.1	1.97	2.75	2.28		5.5
16	23.6	1.84	2.63	2.17		5.1
17	17.3	2.07	2.92	2.46		5.2
Mean	19.2	2.79	3.15	2.44	0.018	7.4
SEM	0.8	0.33	0.32	0.15	0.004	1.1
				(77.5%)		
		4.42 % dose/L				0.00118 % dose/g
		6.36 % dose/Tot Blood Vol				
METHYLPREDNISOLONE						
1	17.3	3.75				7.7
2	17.3	2.84				4.8
3	17.7	1.51				6.4
4	9.1	1.44				14.6
5	18.2	1.45				3.1
6	20.5	2.65				6.0
7	22.7	1.87	2.76		0.015	3.6
8	14.1	3.38	4.69		0.032	8.1
9	18.2	2.30	3.14		0.015	4.4
10	26.4	2.04	3.07		0.012	5.0
11	25.5	1.42	2.14		0.012	3.2
12	22.3	1.32	1.88		0.018	3.6
13	16.8	2.13	3.12	2.41		5.9
14	14.5	4.49	6.85	4.66		9.1
15	26.4	1.20	1.61	1.44		3.1
16	22.3	2.24	3.27	2.35		5.0
17	20.5	1.30	1.69	1.39		3.1
Mean	19.4	2.20	3.11	2.45	0.017	5.0
SEM	0.9	0.23	0.46	0.59	0.003	0.7
				(78.8%)		
		3.49 % dose/L				0.00079 % dose/g
		5.06 % dose/Tot Blood Vol				
	NS	NS	NS	NS	NS	$p < 0.10$

Effects of MP on ^{99m}Tc-PYP Excretion

An explanation for reduced blood and tissue PYP levels in the MP group is provided by analysis of renal clearance data (Table 6). In the 5 pairs of animals given exogenous creatinine, mean plasma creatinine was less and total creatinine excretion was greater in the methylprednisolone-treated group; these changes were not statistically significant because of the great variability in the MP group. Urinary clearance of both creatinine and PYP was greater in the MP group, but again fell short of statistical significance. Interestingly, mean creatinine clearance (^{C}Creatinine) and mean PYP clearance (^{C}PYP), in ml/minute, were equivalent by paired t test analysis in both control and MP groups (Table 6). Linear regression analysis reveals a direct correlation between ^{C}PYP and ^{C}Creatinine, with $r=0.95$ (Figure 9). This suggests that PYP is excreted by a pure glomerular mechanism, and that ^{C}PYP is, like ^{C}Creatinine, an indicator of glomerular filtration rate. This finding is used to good advantage in Table 7, where ^{C}PYP and urine PYP excretion are presented for all animals in which urine output was monitored. Note that one MP dog (8) was eliminated because of urinary leak around the catheter. ^{C}PYP was significantly greater by group t test in the MP group than control, 163.4± 25.7 vs. 111.1± 13.4 ml/minute, $p<0.05$. Fractional excretion of PYP, ^{C}PYP/^{C}Creatinine, an indicator of renal tubular function, did not differ between the two groups of 5 dogs for which this supplementary information is available (Table 6). These data strongly indicate an increase in glomerular filtration rate 24 hours after administration of a single, massive dose of methylprednisolone. The result is that urine PYP excretion was greater in MP animals

TABLE 6 Excretion and Clearance of Creatinine and ^{99m}Tc-PYP

	Plasma Creatinine (mg/100 ml)	Creatinine Excreted (mg)	$C_{Creatinine}$ (ml/min)	C_{PYP} (ml/min)	$\frac{C_{PYP}}{C_{Creatinine}}$
CONTROL					
13	6.21	305.8	82.1	81.5	0.99
14	6.49	318.5	81.8	113.0	1.38
15	6.61	373.0	94.0	119.5	1.27
16	4.16	288.0	115.5	118.6	1.03
17	6.29	332.1	88.0	113.7	1.29
Mean	5.95	323.5	92.3*	109.3*	1.19
SEM	0.45	14.3	6.2	7.1	0.07
METHYLPREDNISOLONE					
13	6.35	287.4	75.4	117.5	1.56
14	5.45	314.1	96.1	72.3	0.75
15	4.92	1156.5	391.8	345.2	0.88
16	3.90	289.0	123.5	109.9	0.89
17	4.95	442.2	148.9	204.4	1.37
Mean	5.11	497.8	167.1**	169.9**	1.09
SEM	0.40	167.1	57.5	48.9	0.16
	NS	NS	NS	NS	NS

* Difference in means NS by paired t test.

** Difference in means NS by paired t test.

TABLE 7 Urine Volume and ^{99m}Tc-PYP Clearance and Excretion

	Urine Volume (ml/ 1 hour)	C_{PYP} (ml/min)	PYP Excretion (% dose/ 1 hour)
CONTROL			
7*	17.1	76.2	26.7
8	7.9	68.4	40.6
9	8.7	52.2	24.4
10*	18.5	118.0	31.2
11*	13.9	217.0	27.6
12	70.7	143.6	40.9
13	71.6	81.5	31.9
14	48.1	113.0	40.0
15	68.8	119.5	44.5
16	292.1	118.6	36.0
17	73.1	113.7	49.7
Mean	62.8	111.1	35.8
SEM	24.4	13.4	2.4
METHYLPREDNISOLONE			
7*	23.9	210.8	62.0
9	236.0	104.5	41.7
10*	45.9	120.7	32.4
11*	22.7	216.4	36.9
12	381.0	131.8	49.6
13	144.6	117.5	35.6
14	396.2	72.3	54.1
15	794.0	345.2	58.5
16	365.5	109.9	38.0
17	411.5	204.4	60.6
Mean	282.1	163.4	46.9
SEM	76.0	25.7	3.6
	$p<0.005$	$p<0.05$	$p<0.01$

* Pairs of animals with similar urine volume, utilized in assessment of blood PYP activity. (See Table 3, and text.)

than control, 46.9± 3.6 vs. 35.8± 2.4% injected dose in one hour, $p<0.01$ (Table 7). These changes in PYP clearance and excretion are depicted graphically in Figure 10.

DISCUSSION

Effects of Methylprednisolone on Countershock Injury

Myocardial ^{99m}Tc-PYP uptake 24 hours following transthoracic DC countershock correlated well with tissue CPK depletion (Figure 2). Myocardial CPK levels are presumed to indicate the degree of cellular injury. In experimental myocardial infarction produced by coronary occlusion in rabbits, there is a linear relationship between regional myocardial blood flow and CPK depletion (120). Quantitative comparisons between tissue CPK concentration and the histologic extent of necrosis are difficult to make. Most attempts to quantify myocardial necrosis histologically have depended largely on the weight or area of tissue showing gross or microscopic evidence of necrosis (19, 104, 106, 120). A semiquantitative histologic grading of myocardial biopsies was used in the current study. However, the biopsies examined showed necrosis of of such similar extent that it was not possible to distinguish among them sufficiently to permit quantitative correlation with either tissue CPK or ^{99m}Tc-PYP levels. Nevertheless, it is felt that myocardial CPK concentration is a meaningful reflection of the extent of tissue injury. The demonstration of a good correlation between tissue CPK depletion and ^{99m}Tc-PYP accumulation 24 hours after countershock was expected because this injury is known to spare blood flow (21), and is the basis for using PYP tissue uptake in a quantitative way to assess myocardial necrosis after countershock.

Dogs treated with a massive dose of methylprednisolone 90 minutes before two 400 watt-second DC countershocks had a higher mean ^{99m}Tc-PYP sample:normal ratio than control, suggesting increased damage (Figure 3).

However, this was associated with reduced normal zone or background myocardial PYP levels, rather than an expected increase in abnormal zone uptake. Assessment of the extent of myocardial injury by tissue CPK depletion, precordial ST segment mapping and histologic grading provided additional evidence that methylprednisolone did not modify countershock damage.

Absolute normal zone PYP activity was significantly less in the MP group than control. This could reflect alterations in any factor capable of lowering circulating PYP, the most likely possibilities being increased bone uptake or increased urinary excretion. One may postulate that accumulation of PYP by necrotic myocardium occurs rapidly after injection of tracer. If most PYP uptake occurs during the first minutes after injection, before blood levels have been affected by different rates of bone uptake or urinary excretion, then abnormal zone PYP uptake would not be altered by changes in radionuclide kinetics. In this context it is worth considering that the major portion of myocardial uptake of potassium-43 and thallium-201, radioactive cations which accumulate in normal tissue and leave infarcted tissue as a filling defect or a "cold spot", does occur during the first minute after injection (121).

Radionuclide image detection depends on relative activity differences between regions of increased or decreased uptake and background. This is the rationale behind expressing tissue radionuclide uptake in terms of a sample:normal ratio. In addition, variability in absolute abnormal zone count rates is reduced by normalizing to background or normal zone activity. The data presented are instructive, however, in that by reducing normal zone PYP activity, methylprednisolone treatment lowered the setting of the background, and made the sample:normal ratio

unreliable as an expression of abnormal zone radionuclide uptake.

Tissue CPK assessment of countershock damage was limited to 5 control and 4 MP dogs because of the previously demonstrated correlation between CPK depletion and PYP uptake (Figure 2). The available data reveal no difference between the two groups (Figure 5). Mean absolute normal zone CPK levels were similar in control and MP animals, with a fair amount of inherent variability. Thus, the CPK sample:normal ratio was a valid expression of tissue enzyme activity.

Precordial ST segment mapping revealed no difference in ΣST or NST between the two groups at 0, 5 or 60 minutes (Figure 6). ΣST is considered to indicate the severity of myocardial damage, while NST reflects the area of injury (25). Several animals in both groups with sizable abnormal zones had minimal or absent ST elevation, indicating that ST segment analysis is an insensitive indicator of myocardial damage in the countershock model. Correlation of ST segment data with PYP data was poor, with the best correlation, r= 0.40, obtained by comparing ΣST at 5 minutes and the PYP sample:normal ratio. ST segment mapping was not a dependable means of quantifying myocardial injury in this model, even after eliminating animals without substantial ST elevations. In contrast to our results, Dahl et al. were able to show an excellent correlation (r= 0.89) between mean ST segment elevation, obtained by means of a 25 lead precordial grid, and a "myocardial damage index", derived from gross area and microscopic severity of necrosis, in 42 dogs after ten transthoracic countershocks (19). They also showed that ST elevation increased as the size of paddle electrodes and the time interval between shocks decreased. These authors do not report the length of time between termination of the discharges and ST mapping. More

importantly, they report that 16 animals had myocardial necrosis without ST elevation. Inclusion of these 16 animals in linear regression analysis would presumably have yielded a less impressive correlation between ST segment elevation and the "myocardial damage index".

Epicardial (19) and precordial (81,122) ST segment mapping have been demonstrated to be a useful quantitative means of evaluating myocardial ischemic injury and its pharmacologic and hemodynamic modification. However, recent reviews have emphasized a number of limitations, particularly in the clinical setting (123-125). ST segment changes result from electrical interactions between normal and abnormal tissue, and may be altered by factors modifying membrane potentials in either area (125). Precordial ST segment mapping, in particular, is limited by the electrical resistance of the chest wall and by the distance and three-dimensional geometry of the zone of injury (125). Characterization of countershock-produced myocardial damage by precordial ST segment analysis is limited by these factors, with the additional geometric problem of two distinct zones of injury on opposite sides of the heart.

The evidence presented indicates that corticosteroid pretreatment failed to modify countershock injury. Potential explanations might be: a) that corticosteroid as administered in these studies does not reduce myocardial cell death; b) that the electrical energy applied was too great to permit salvage of myocardium; and/or c) that countershock necrosis differs from ischemic necrosis in some way affecting potential for tissue salvage.

None of the several mechanisms by which corticosteroids are believed to modify myocardial injury is beyond reasonable doubt, as discussed in the review of the literature. Steroids certainly stabilize

lysosomal membranes in vitro, but may not in vivo. There is no evidence that the anti-inflammatory effects of corticosteroids reduce myocardial cell destruction. The hemodynamic and metabolic effects of corticosteroids have been studied intensively, without proof of long term benefit, i.e., reduced infarct size. Corticosteroids appear to increase coronary blood flow, an effect of obvious importance to the ischemic myocardium. Coronary blood flow was not measured in our experiments. Regional myocardial blood flow 24 hours after countershock is normal- i.e., there is no ischemic zone (21). Even in the absence of an "ischemic stimulus" (73), it is possible that methylprednisolone treatment did increase myocardial blood flow. If so, there would have been increased delivery of ^{99m}Tc-PYP to hearts of MP animals, and one would have expected greater myocardial radionuclide uptake in the MP group. In fact, abnormal zone PYP uptake was not significantly different in the two groups, and normal zone PYP activity was clearly less in MP dogs than control. This indicates that if coronary blood flow had not been increased by methylprednisolone, then myocardial PYP uptake might have been even less in the MP group. In other words, augmented coronary blood flow, if present, opposed and minimized the effect noted.

Maintenance of regional myocardial blood flow is not the only difference between countershock injury and ischemic injury. Myocardial infarction is not a sudden, all or none event. Jennings et al. showed that after coronary occlusion in the dog, the first irreversibly damaged cells appear at 22 minutes post-occlusion, and that even after 45 minutes, 35-66% of the cells remain viable (24). The fact that all ischemic cells do not die is the obvious basis for measures aimed at salvaging reversibly injured myocardium, as previously discussed. Evidence of early,

reversible cellular injury is noted by electron microscopy after 5 to 15 minutes of acute experimental ischemia, and by light microscopy after 20 to 30 minutes. A number of morphologic and metabolic stages of early cellular injury are described (86, 87, 126) There is no sharp line dividing reversible from irreversible injury. Recent studies by Jennings' group indicate that after 40 minutes of experimental myocardial ischemia, there is loss of plasma membrane integrity, with inability to maintain intracellular volume and electrolyte composition (127), associated morphologically with cell swelling, muscle fiber contraction bands, and formation of amorphous densities in mitochondria staining positively for calcium (128), and associated metabolically with defective mitochondrial respiratory function (129). Such changes are not noted after 15 minutes of ischemia, and are felt to indicate irreversible damage.

Certain non-ischemic cellular injuries may result from direct attack on the cell membrane, causing rapid cell death and lysis (126). Antibody and complement, ultraviolet radiation and direct mechanical injury may destroy cells in this manner. Such agents might exert their cytotoxic effects in a rapid, all or none fashion. If DC countershock acts in like manner to produce myocardial necrosis, then its effects may not be modifiable by the subtle benefits ofcorticosteroid intervention. Perhaps, also, the electrical energy applied was too great to permit significant modification of damage; lesser energies might conceivably have left more salvageable myocardium.

Tedeschi and White initially described the areas of cardiac necrosis produced by epicardially applied countershock as "burns", and identified characteristic vascular engorgement, hemorrhage and edema, and "regressive" changes in muscle fibers (16). Anderson et al.found that the temperature

of countershock lesions, measured with thermistor probes, was normal, suggesting that the damage was electrical, not a true burn (17). They also described the following sequence of histological changes post-countershock: immediately, loss of cytoplasmic organization, transverse banding in myofibrils, interstitial edema without cellular reaction; at 3 days, interstitial infiltrate composed of endothelial cells, macrophages, fibroblasts and very few polymorphonuclear leukocytes; at 1 week, increased cellular reaction; from 2 to 4 weeks, disappearance of cellular reaction, increasing fibrosis. The lesions as described differ from ischemic coagulation necrosis in two important ways: 1) immediate cytoplasmic changes; and 2) few polymorphonuclear leukocytes (17). The virtual absence of polys and predominence of mononuclear cells and fibroblasts has been confirmed in transthoracic countershock (18, 20).

Reichenbach and Benditt believe that the histologic changes associated with countershock fit into the pattern of myocardial injury known as "myofibrillar degeneration" (130). This cardiac lesion occurs, as does countershock, in the presence of an intact microcirculation. It is reportedly a common autopsy finding in man, and accompanies various clinical and experimental situations. In particular, it is associated with catecholamine excess, both endogenous (pheochromocytoma in man) and exogenous (isoproterenol-induced cardiac injury in rabbits). It is postulated that myofibrillar degeneration is the result of a common pathogenic mechanism whereby catecholamines released locally by myocardial sympathetic nerve terminals or distantly by the adrenal glands increase cardiac work and oxygen requirements while uncoupling oxidative-phosphorylation and reducing available ATP, resulting in myocardial cellular necrosis (130).

Davis et al. have looked again at the morphology of countershock-produced myocardial injury, and feel that it is, indeed, a form of myofibrillar degeneration (18). After several weeks the fibrous scar of countershock-produced necrosis is indistinguishable from that of ischemic infarction, but they appear to originate as distinctly different forms of injury. This could at least in part, explain different responses to corticosteroid pharmacologic intervention. In this context, it is interesting that Fleckenstein has reported that pretreatment with 9α-fluorocortisol markedly increases isoproterenol-induced myocardial necrosis and calcium uptake in rats (99). This, then, is a situation in which corticosteroid exacerbates myofibrillar degeneration.

Effects of Methylprednisolone on ^{99m}Tc-PYP Distribution and Excretion

The data presented suggest that reduced normal or background myocardial ^{99m}Tc-pyrophosphate activity in methylprednisolone-treated dogs was related to reduced circulating PYP. It seems likely that what is measured in the scintillation counter as "uptake" of radionuclide by normal myocardium and other non-osseous tissues is actually extracellular radionuclide in capillary blood. As noted, presumed differences in intravascular volume in the two groups, resulting from markedly different urine output after water loading, probably masked the expected difference in blood PYP activity. Even so, a trend was suggested where blood clearance of PYP was more rapid, i.e., PYP levels became less, in the methylprednisolone-treated group than control (Figure 8). Considering only animals not water loaded, or with comparable urine output, blood PYP was significantly less in the MP group than control at the time of sacrifice (Table 3).

Blood ^{99m}Tc-PYP activity was expressed here as absolute counts per minute (CPM)/ml/mCi injected (Table 3) and not as a percentage of the injected dose because well-counter calibration - saving and counting fractions of a 1:1000 dilution of a 0.1 ml aliquot of the dose to permit calculation of CPM/mCi injected - was done only in the last 11 pairs of animals studied. The value for CPM/mCi varied somewhat from day to day, perhaps due to well-counter voltage fluxes, averaging 6.31×10^8 CPM/mCi (range: 5.37 to 8.19×10^8 CPM/mCi). Thus, if 10 mCi of ^{99m}Tc-PYP were administered to all animals, then the approximate average dose would be 6.31×10^9 CPM. For each time of injection, expression of blood PYP activity (in CPM/ml/mCi) as a percentage of the approximate total dose injected (6.31×10^9 CPM/ 10 mCi), times 1000, gives an estimation of the mean % injected dose per liter of whole blood (Table 3). Assuming that total blood volume in the dog approximates 7.5% of body weight in liters, and using the mean body weights for the two groups of dogs given in Table 5, these data can be converted to mean % injected dose per total blood volume (Table 3). It thus is estimated that in the control group, 4.4% dose/liter whole blood (or 6.3% dose/ total blood volume) circulated at 60 minutes after injection. In the methylprednisolone group, the values are somewhat lower - 3.5% dose/L (or 5.1% dose/ total blood volume). Considering, as before, animals not water loaded or with similar urine volume, the estimated values become: control, 5.2% dose/L (or 7.5% dose/total blood volume), and methylprednisolone, 3.3% dose/L (or 4.8% dose/total blood volume).

These approximations compare well with those of others who have studied blood clearance of ^{99m}Tc-PYP. At one hour, Hosain measured about 5% of the injected dose in the total blood volume of 5 dogs (131). Krish-

namurthy et al. found 2.9% dose/L whole blood in 10 patients at one hour (133). Subramanian et al. reported 13.0% dose/total blood volume at one hour in 6 patients (132), which, assuming 70 kg and a total blood volume of 7% body weight in liters, converts to 2.7% dose/L.

In our experiments, binding of ^{99m}Tc-PYP to plasma proteins did not differ in the two small groups studied. A reduction in plasma protein-binding in the methylprednisolone group could have accounted for more rapid urinary excretion secondary to increased filtered load. Protein-bound PYP represented 77.5% of plasma activity in the control group and 78.8% of plasma activity in the MP group (figure 9). Others have reported the percentage of plasma protein-bound PYP at one hour after injection to be 84.3% in the dog (133), and 45.4% (132) and from 10-69% (135) in man. ^{99m}Tc-PYP binds loosely, and mainly to the globulin fraction (133).

Bone PYP uptake was also similar in the two groups of dogs, averaging .018% dose/gm in the control group and .017% dose/gm in the MP group (Table 5). ^{99m}Tc-PYP is a bone-seeking radionuclide, and bone is the body's principal PYP reservoir (131-133). Thus, increased bone PYP uptake in the methylprednisolone group could have accounted for reduced circulating PYP and reduced normal myocardial PYP. Bone PYP count density in our studies approximates that reported by Bonte et al. in early experiments (136, 137). Eckelman et al. found PYP uptake of .006% dose/gm in dog femur (138). We have noted considerable variation in percentage uptake of PYP dose by rib, iliac crest and femur in individual animals, a fact observed by others using a number of skeletal imaging agents (132).

Myocardial PYP accumulation was less in the methylprednisolone-

treated group, averaging .00079% dose/gm vs. .00118% dose/gm in the control group. Bonte et al. found normal myocardial PYP activity at one hour to average .00077% dose/gm (136) and .0028% dose/gm (137) in separate experiments with small numbers of dogs.

Kinetic studies by Krishnamurthy et al. have indicated that clearance of ^{99m}Tc-PYP from the blood occurs biexponentially with the first phase thought to represent bone uptake and having a half-life ($T_{1/2}$) of 13.6 minutes, and the second phase believed to indicate renal clearance and having a $T_{1/2}$ of over 6 hours (133). Their data indicated that by one hour, in man, 16.4% of the injected PYP dose was excreted in the urine. Others have reported higher values for one hour urine PYP excretion amounting to 15-35% dose (135) and 29.9% dose (132) in man, and nearly 35% dose in the dog (131). Our control dogs had a mean one hour urine PYP excretion of 35.8% of the injected dose, compared with a significantly greater PYP excretion of 46.9% dose in methylprednisolone-treated animals (Table 7). This represents a 31% increase in one hour urinary PYP excretion occurring 24 hours after massive, single dose methylprednisolone treatment. It appears to be sufficient to explain reduced blood and background myocardial PYP levels in these animals. Further analysis of data reveals the mechanism by which methylprednisolone treatment increased urinary PYP excretion.

The demonstration of a close correlation between clearance of PYP and creatinine clearance in 10 dogs (Figure 9) suggests that, like inulin and creatinine in the dog, PYP is excreted by a pure glomerular mechanism. PYP clearance was significantly greater in the methylprednisolone-treated group than control (Table 7). Fractional excretion of PYP, an index of renal tubular function, was the same in both groups. These findings

indicate an increase in glomerular filtration rate 24 hours after administration of a single large dose of methylprednisolone.

There are a number of precedents for an increase in GFR after several days of adrenocorticotropic hormone (ACTH) or corticosteroid administration to small groups of patients with normal or impaired renal function (139-142). In studies by Levitt and Bader in 6 patients, inulin clearance increased an average of 31% progressively during ACTH or cortisone administration over a week or more (142). Patients reported by Alexander et al. had increments in inulin clearance as great as 103% and 86% after ACTH treatment for 8 and 14 days, respectively (141). Chronic administration of ACTH or cortisone to four dogs increased creatinine clearance, twice after only one dose (143). Acute administration of dexamethasone, 8-10 mg IV, to four dogs had an immediate effect of GFR, which increased a mean of 29% (range 5-45%) by one hour (144). This effect of corticosteroids was in one study (142) clearly associated with expansion of extracellular fluid volume, but in others (143,144), it appeared to occur independently. Methylprednisolone, which has little or no mineralocorticoid effect (145), has been shown to increase inulin clearance in the dog (146) and in the rat (147) with chronic administration. The mechanism by which glucocorticoids increase GFR is not entirely clear, but several studies have demonstrated an associated increase in renal plasma flow (139, 140, 143, 147). DeBermudez and Hayslett showed with redioactive microspheres that the 42% increase in renal plasma flow after methylprednisolone administration in rats occurred predominently in the inner cortex, where nephrons with long loops of Henle may play a special role in sodium reabsorption (147). The redistribution of renal blood flow to the inner cortex may not explain the increase in glomerular

filtration rate, but it would provide a means of maintaining sodium balance through increased tubular reabsorption in the face of an increased filtered load.

Changes in the urinary excretion of a variety of substances occur following the administration of ACTH or cortisone. After several days of such therapy in patients, there may be increased urinary excretion of potassium (148), uric acid (139, 148) and phosphate (140, 148), and decreased urinary excretion of sodium (141, 148). These effects appear to reflect alterations in renal tubular transport of filtered substances (148). In our studies, renal tubular handling of ^{99m}Tc-PYP appeared to be unchanged, supporting a primary effect of methylprednisolone on glomerular filtration rate.

An incidental observation in the present study is the massive increase in urine volume in the MP group. Several reports confirm an increase in free water clearance after corticosteroid treatment (143, 146, 147, 149). Patients with adrenal insufficiency and adrenalectomized or hypophysectomized animals exhibit impaired free water excretion which is corrected by corticosteroid replacement. Corticosteroids may inhibit the back diffusion of water in the renal tubular diluting segment or collecting duct (149).

The result of increased glomerular filtration rate in the methylprednisolone-treated group was more rapid urinary excretion of PYP, causing lowered blood PYP levels. Since tissue PYP levels appear to reflect blood PYP levels, normal myocardial PYP activity was lower in the MP group than control. It is clear that altered radionuclide kinetics may significantly modify assessment of myocardial injury. This finding is of importance clinically as well as experimentally. Recently, a number

of cases of diffusely positive ^{99m}Tc-PYP myocardial infarct images have been reported in patients because of delayed removal of radionuclide from the blood pool (150, 151). Canine gracilis muscle uptake of potassium-43 and thallium-201 is altered by propanolol and isoproterenol treatment, suggesting that myocardial uptake of these radionuclides might also be changed by commonly employed drug therapy (121). The present experiments confirm that if myocardial radionuclide uptake is to be used to identify myocardial necrosis and to assess its possible modification,then the intrinsic effects of any intervention or other circumstances must be defined to avoid misinterpretation.

Conclusions

Although methylprednisolone-treated dogs had a higher mean ^{99m}Tc-PYP sample:normal ratio, suggesting increased damage over control, this was a result of reduced normal or background myocardial PYP levels. There was no true difference in tissue damage, as assessed by absolute abnormal zone PYP activity, by CPK depletion, by ST segment mapping, and by histopathologic grading.

Potential explanations for the failure of corticosteroid pretreatment to modify countershock injury have been discussed. It is suggested that myocardial necrosis after countershock resembles "myofibrillar degeneration", a lesion distinctly different from ischemic coagulation necrosis. Alternatively, administration of corticosteroids in different dosage or at a different time, or use of lower energy countershock, might have permitted tissue protection.

Reduced normal or background myocardial ^{99m}Tc-PYP in methylprednisolone-treated animals appears to reflect reduced circulating PYP. PYP

distribution studies revealed no difference in plasma protein binding or in bone uptake of PYP. Urinary clearance of PYP, shown to correlate well with creatinine clearance, was significantly greater in the MP group than control. This suggests an increase in glomerular filtration rate after corticosteroid administration, a finding supported by a number of previous studies. As a result, urinary PYP excretion was increased in MP animals, explaining reduced normal myocardial PYP levels. It is concluded that altered radionuclide kinetics may result in incorrect assessment of myocardial injury.

SUMMARY

After a review of pertinent literature, experiments have been described which demonstrate that myocardium damaged by DC countershock in dogs accumulates ^{99m}Tc-pyrophosphate in proportion to the extent of CPK depletion. Pretreatment with methylprednisolone, a corticosteroid, produced no demonstrable difference in tissue damage, as assessed by abnormal zone ^{99m}Tc-pyrophosphate uptake, by tissue CPK depletion, by precordial ST segment mapping, and by histologic and histochemical examination. However, methylprednisolone administration did alter the radionuclide assessment of myocardial necrosis, creating the initial impression of increased tissue damage. Radionuclide distribution and excretion studies indicated that methylprednisolone increased glomerular filtration rate, resulting in accelerated renal excretion of ^{99m}Tc-pyrophosphate. To avoid misinterpretation in the use of myocardial radionuclide uptake in assessing myocardial necrosis, the intrinsic effects of any intervention or other circumstances must be defined.

FIGURES

1. Representative myocardial scan showing two zones of increased ^{99m}Tc-pyrophosphate uptake (a.). After elimination of background activity, abnormal zone was divided by itself to obtain a unity image (b.). Computerized data processing permitted expression of abnormal activity as count density. See text for explanation.

2. ^{99m}Tc-pyrophosphate (PYP) uptake vs. creatine phosphokinase (CPK) depletion, expressed as sample:normal (S:N) ratios, for 50 transmural myocardial samples from areas of countershock injury in three control dogs.

3. Myocardial ^{99m}Tc-PYP tissue uptake in control (C) and methylprednisolone (MP) groups: at left, mean sample:normal ratios; center, mean absolute abnormal zone activity; right, mean absolute normal zone activity. Statistical analysis by group t test. NS = not significant.

4. Abnormal zone size, as assessed by ^{99m}Tc-PYP uptake: at left, mean summed weights of all tissue samples with S:N ratios greater than 3:1; at right, mean radionuclide count density as measured by quantitative imaging as described in text.

5. Myocardial CPK activity in control (C) and methylprednisolone (MP) groups: at left, mean sample:normal ratios; center, mean absolute abnormal zone CPK; at right, mean absolute normal zone CPK. All comparisons not significant (NS).

6. Precordial ST segment mapping: mean ΣST and NST for 14 control and 12 methylprednisolone treated dogs before (0) and 5 and 60 minutes after countershocks. All comparisons NS.

7. Histopathology: a, mean histopathologic grades for 13 biopsies from 8 dogs in each group; b, representative low power (125X) hematoxylin and eosin stained photomicrograph demonstrating fragmenta-

tion of muscle fibers, interstitial edema, and mixed leukocyte reaction; c, subepicardial zone of damaged myocardium accumulating Masson stain; d, high power (500X) hematoxylin and eosin stained section showing obvious myocardial fiber destruction, transverse banding, and cellular reaction, with mononuclear leukocytes predominating over polymorphonuclear cells.

8. ^{99m}Tc-PYP blood clearance curves for control (C) and methylprednisolone (MP) groups. Differences in mean activity at each time after injection of dose are NS, except when animals not water-loaded or with similar urine output are compared (*). Difference between means for these 9 C and 9 MP dogs is significant, $p < 0.025$. See text for explanation.

9. ^{99m}Tc-PYP clearance vs. creatinine clearance in 5 control (C) and 5 methylprednisolone (MP) dogs. Demonstration of a direct correlation suggests that PYP depends largely on glomerular filtration for urinary excretion.

10. ^{99m}Tc-PYP mean clearance (left) and one hour urinary excretion (right) in C and MP groups. Both means are significantly less in the methylprednisolone treated group.

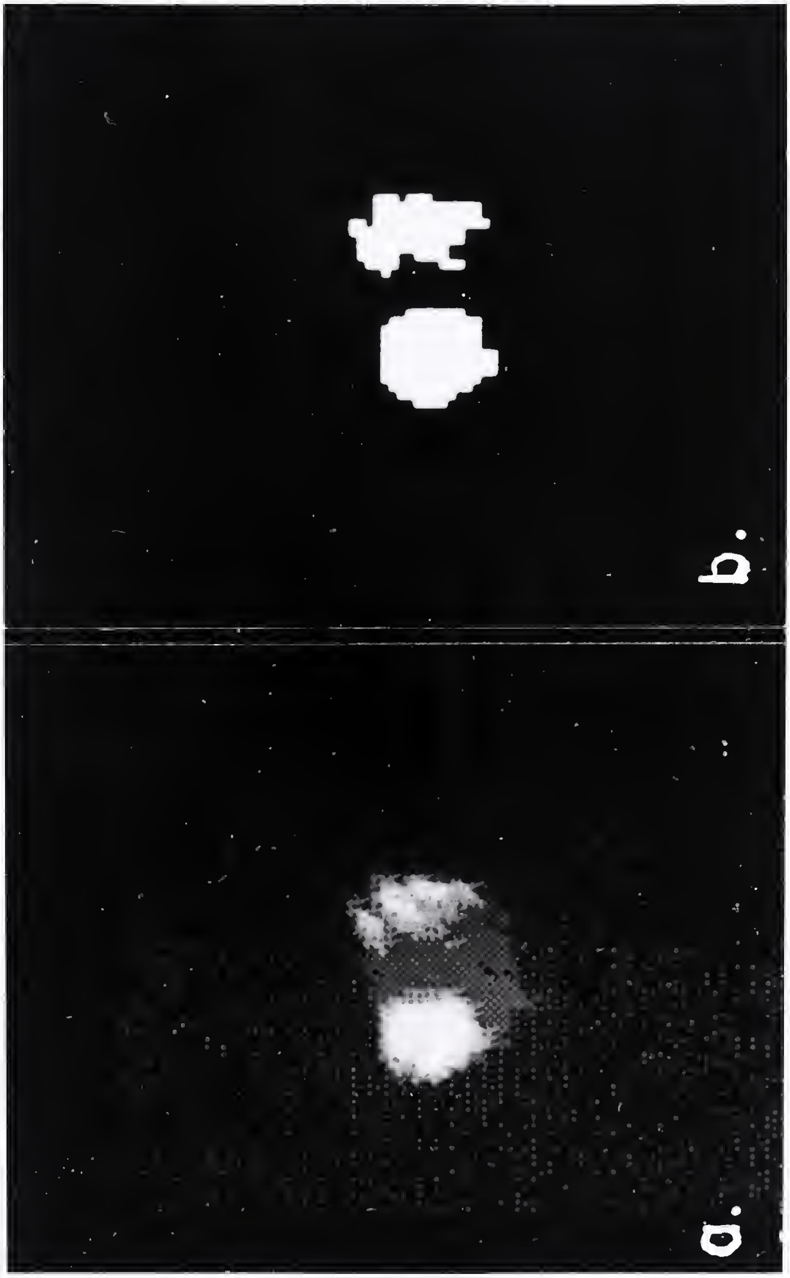


FIGURE 1

D.C. COUNTERSHOCK MODEL

(PYP UPTAKE *vs* CPK DEPLETION)

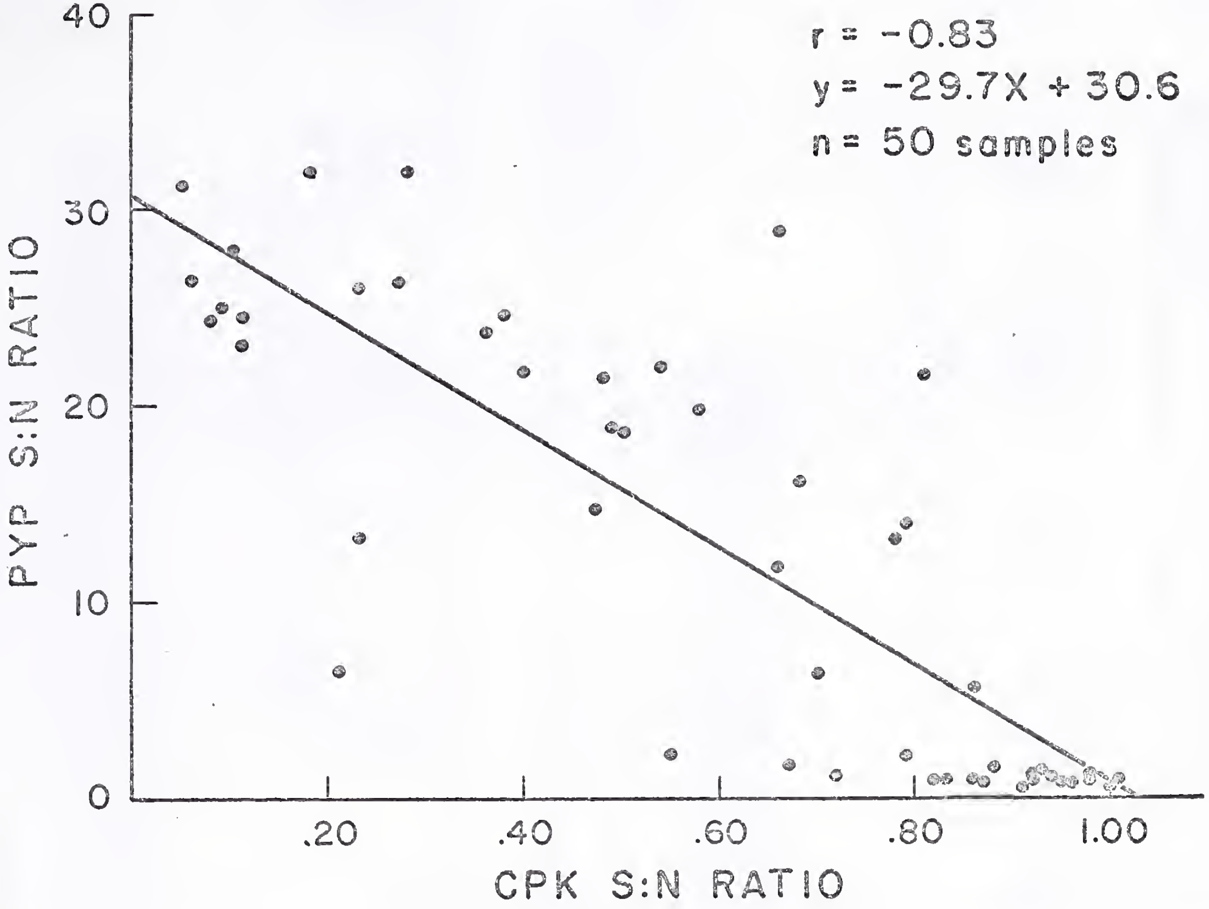


FIGURE 2

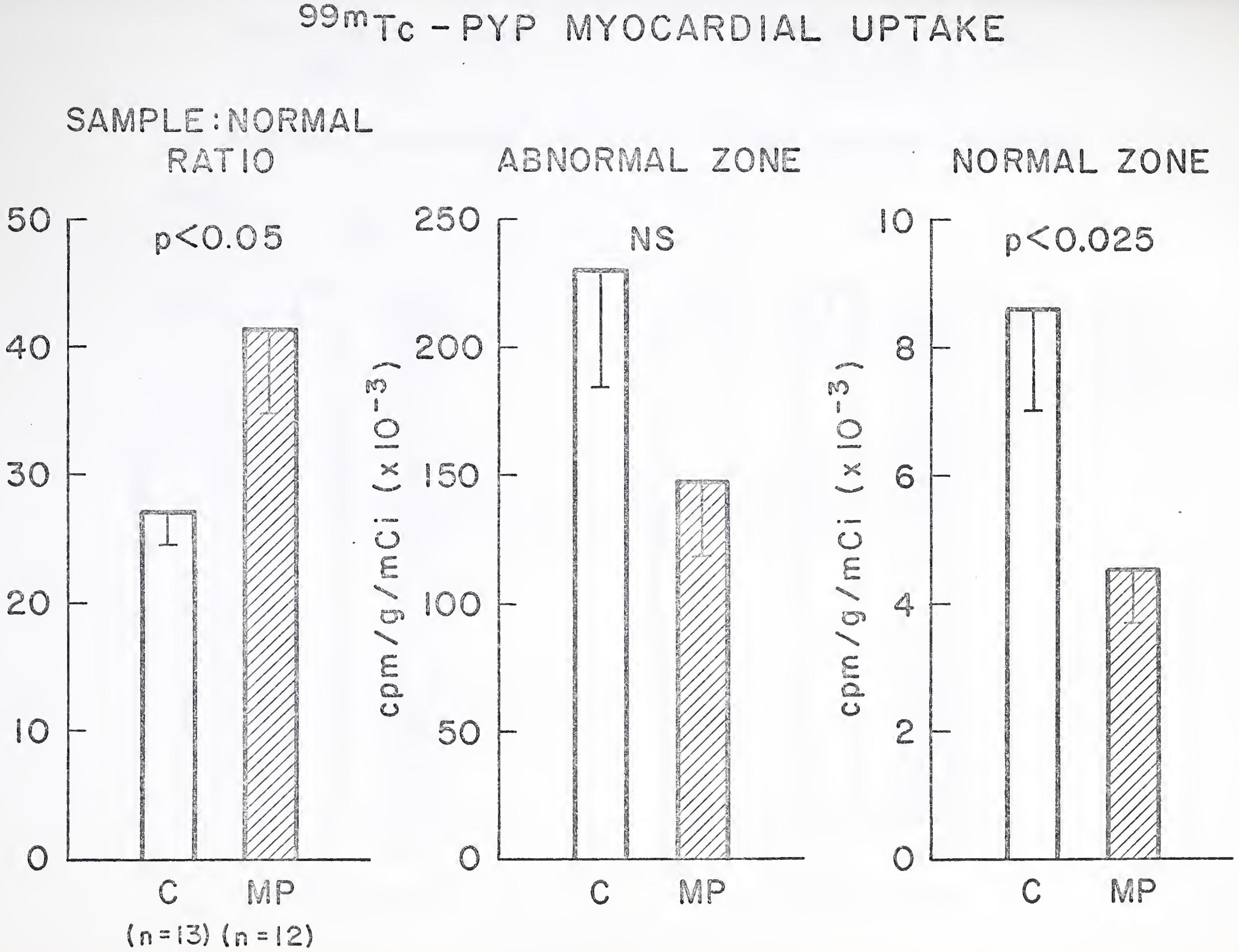

^{99m}Tc - PYP MYOCARDIAL UPTAKE
SAMPLE:NORMAL RATIO
p<0.05
50
40
30
20
10
0
C
MP
(n=13) (n=12)
ABNORMAL ZONE
NS
250
200
150
100
50
0
cpm/g/mCi (x10^{-3})
C
MP
NORMAL ZONE
p<0.025
10
8
6
4
2
0
cpm/g/mCi (x10^{-3})
C
MP

FIGURE 3

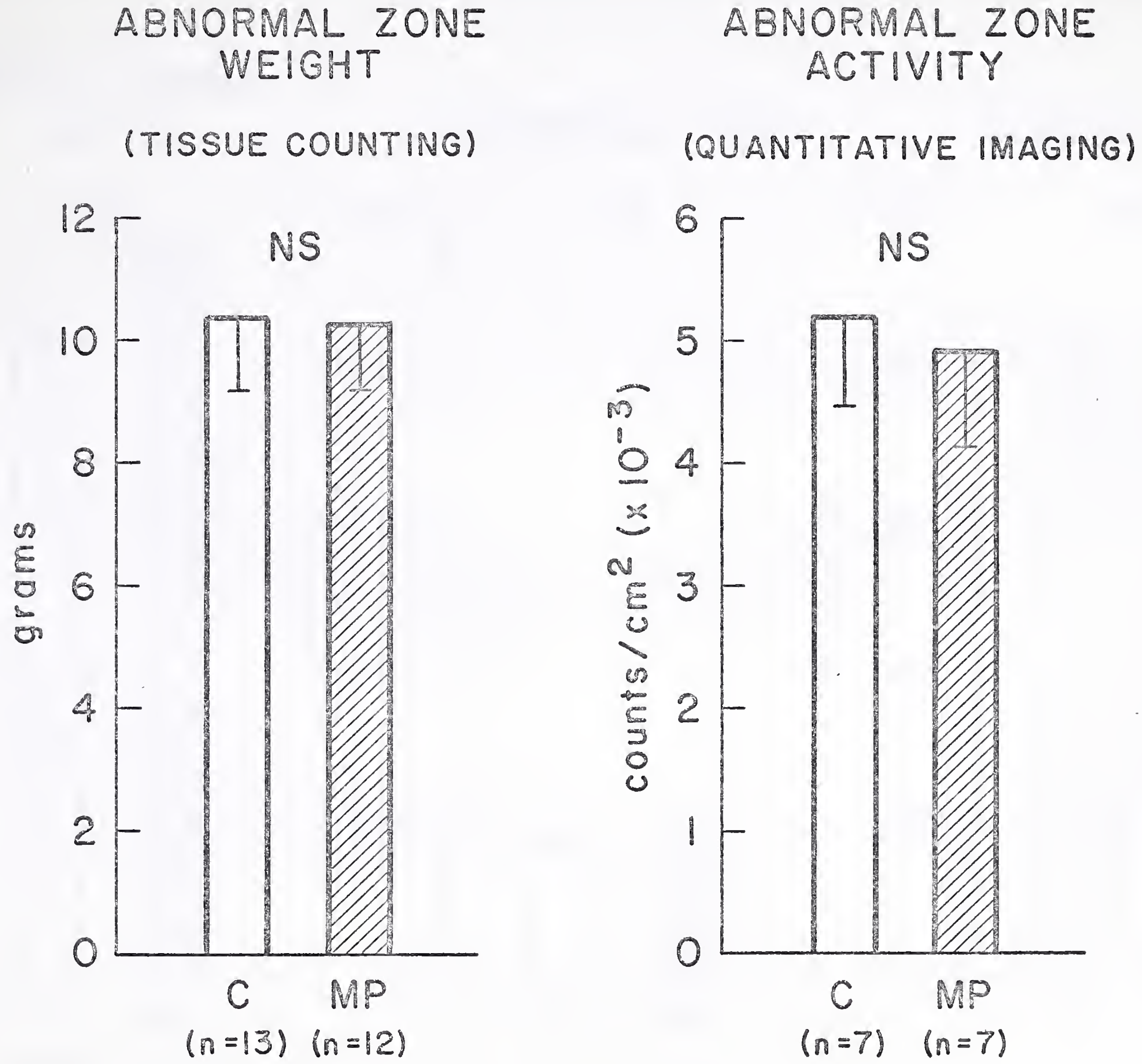

ABNORMAL ZONE WEIGHT
(TISSUE COUNTING)
grams
12
10
8
6
4
2
0
NS
C
(n=13)
MP
(n=12)
ABNORMAL ZONE ACTIVITY
(QUANTITATIVE IMAGING)
counts/cm² (x 10⁻³)
6
5
4
3
2
1
0
NS
C
(n=7)
MP
(n=7)

FIGURE 4

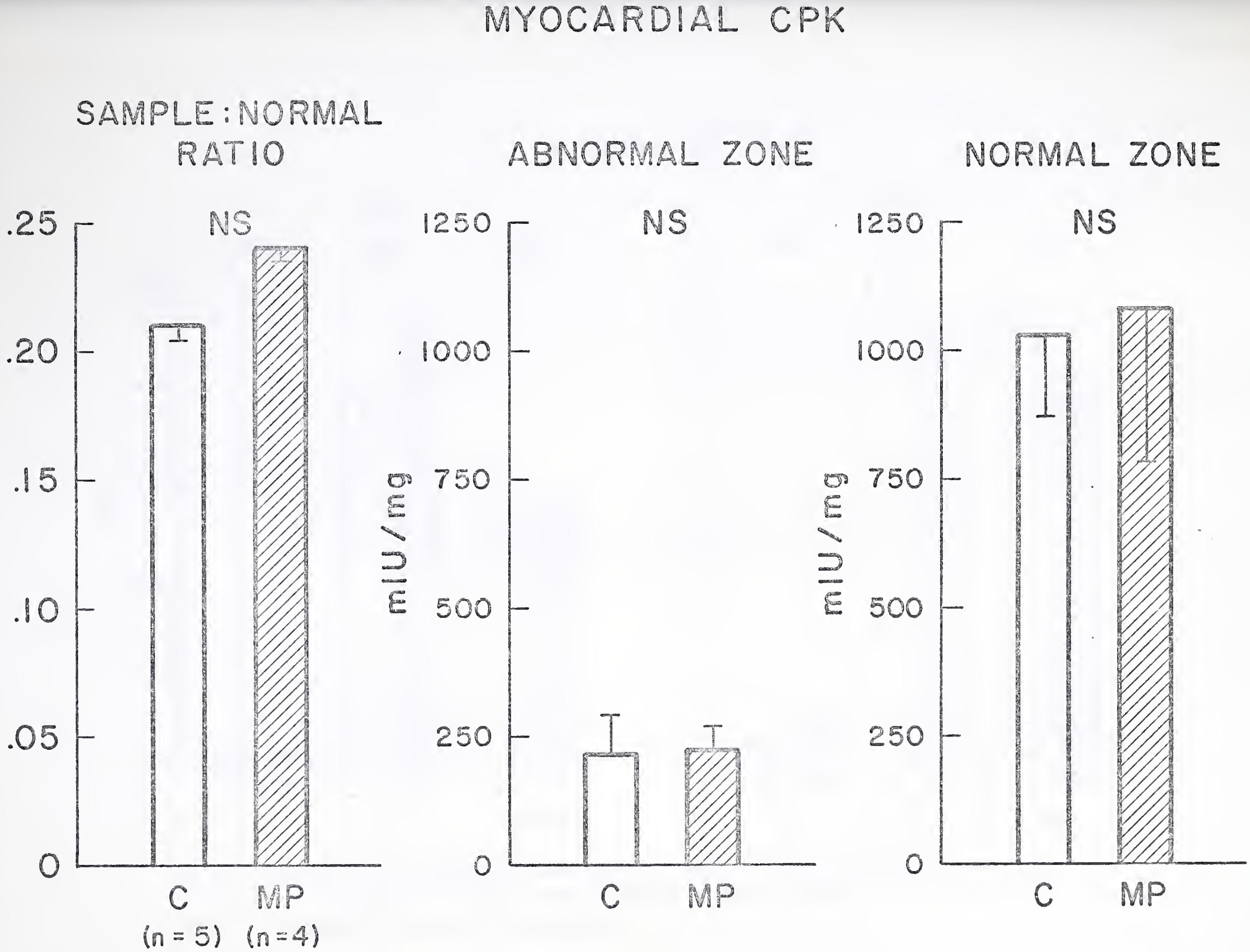
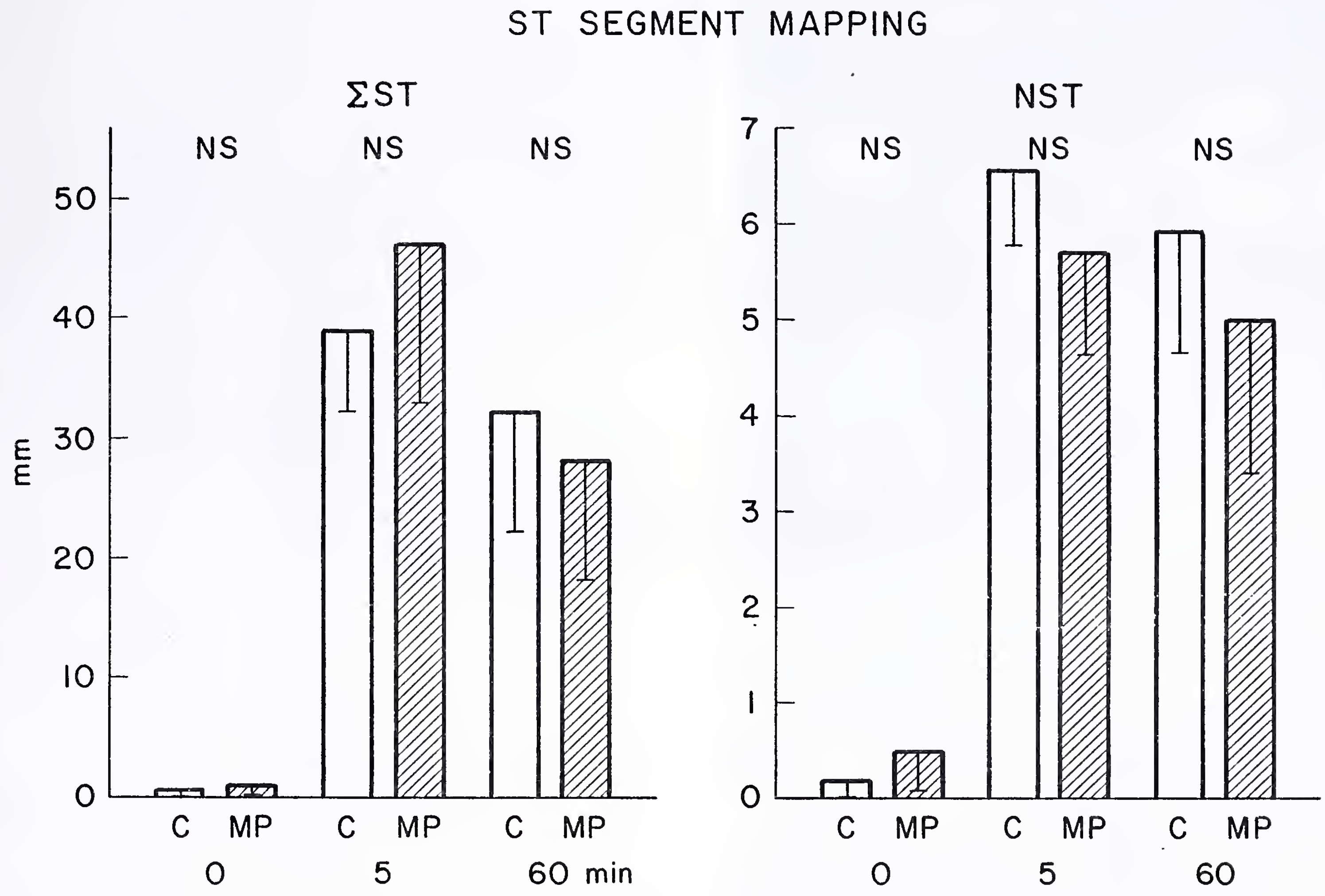

MYOCARDIAL CPK
SAMPLE:NORMAL RATIO
ABNORMAL ZONE
NORMAL ZONE
NS
NS
NS
.25
.20
.15
.10
.05
0
1250
1000
750
500
250
0
mIU/mg
1250
1000
750
500
250
0
mIU/mg
C
MP
(n = 5)
(n = 4)
C
MP
C
MP

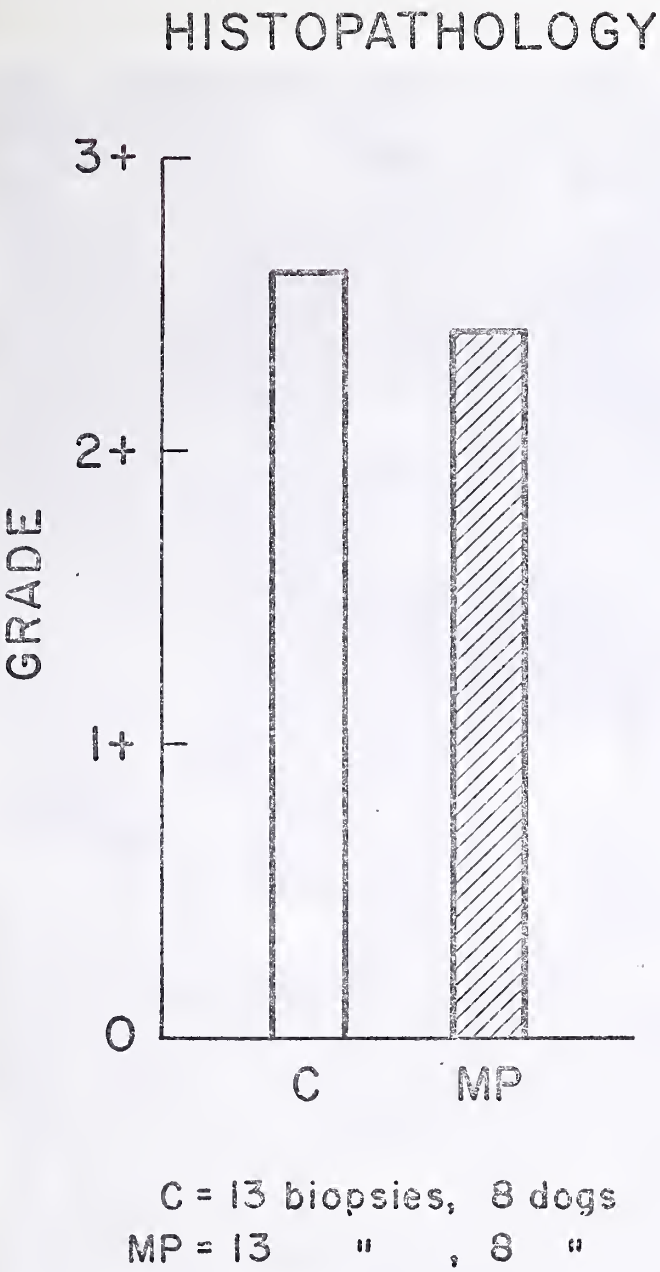
FIGURE 5


ST SEGMENT MAPPING

ΣST = sum of ST segment amplitudes at least 2 mm (0.2 mV) above the PR segment at 0.08 seconds after onset of QRS

NST = number of such ST segments

FIGURE 6



A

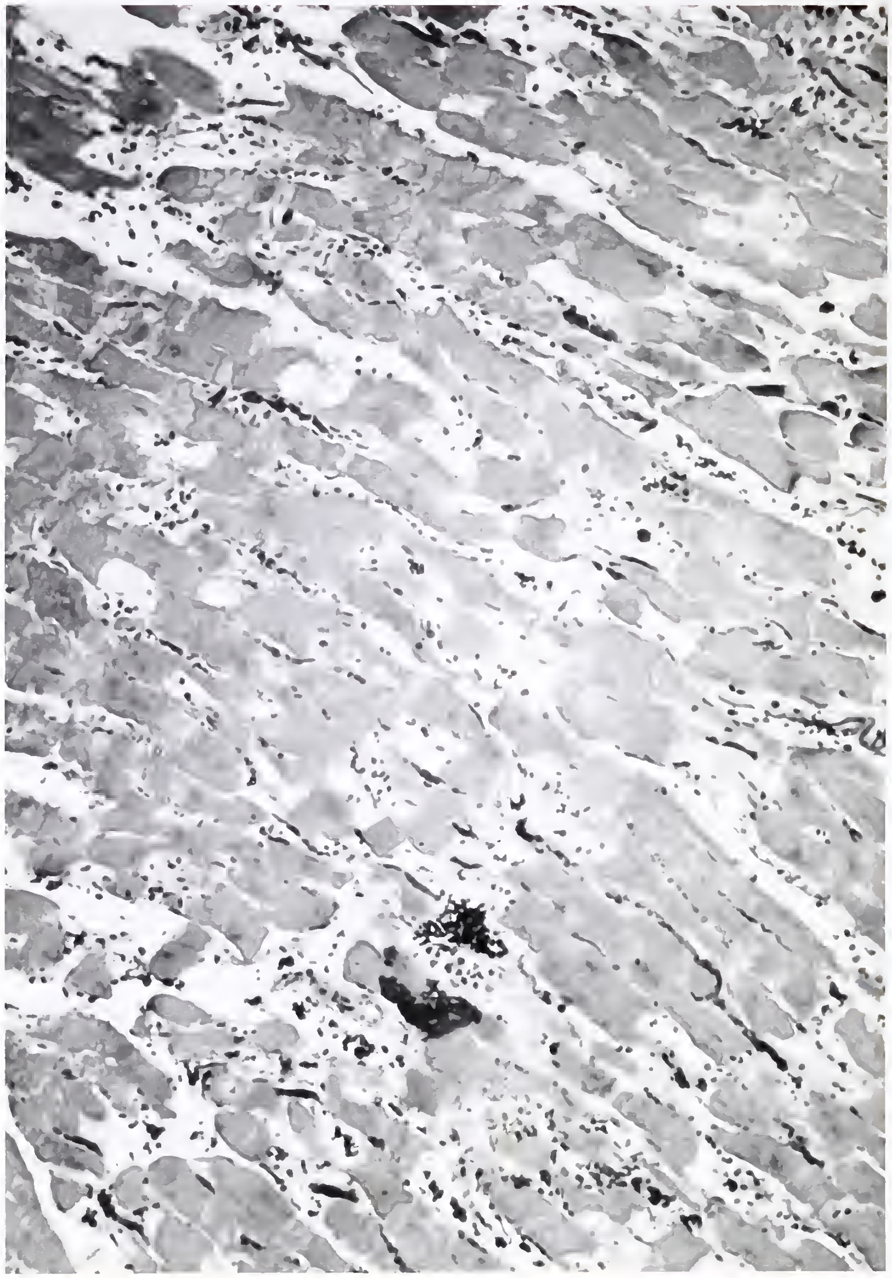
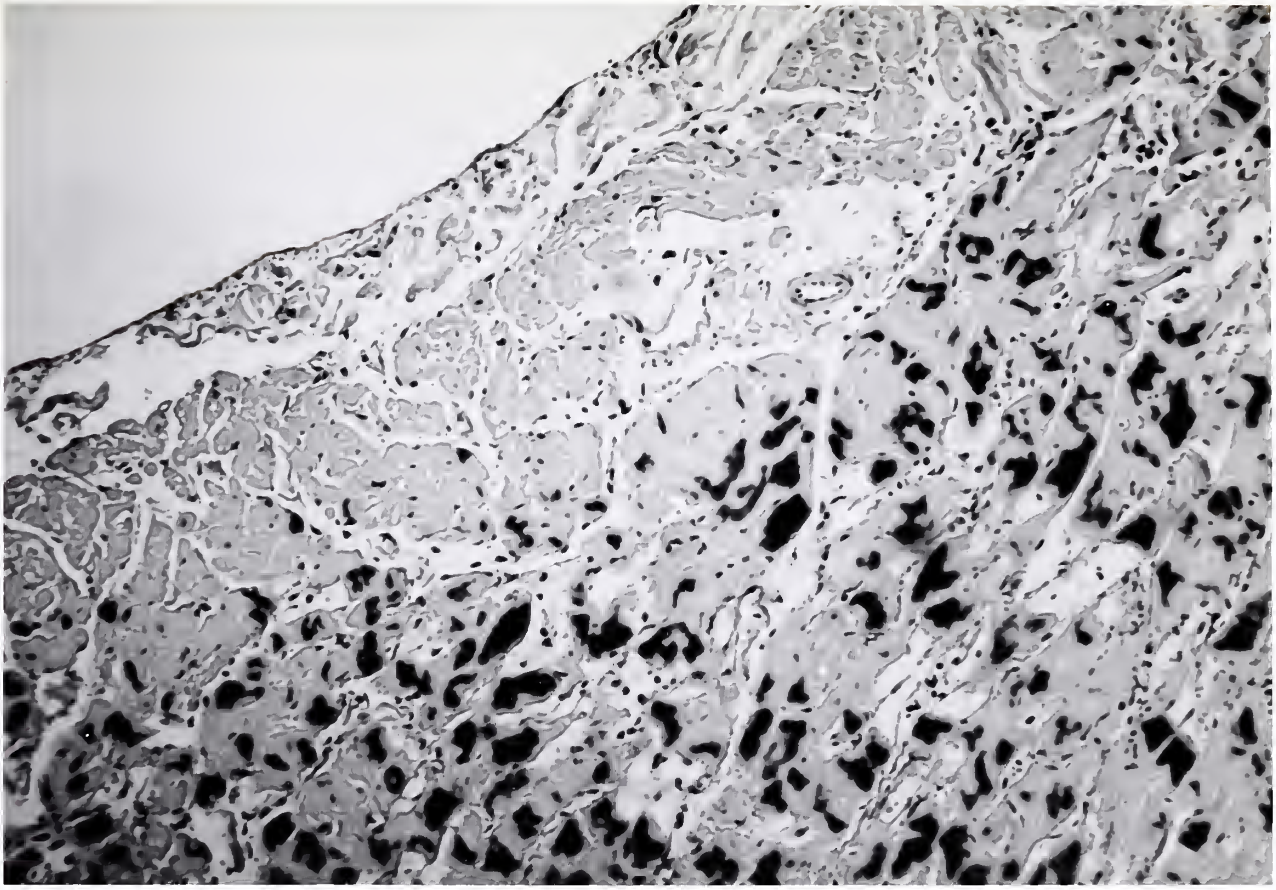
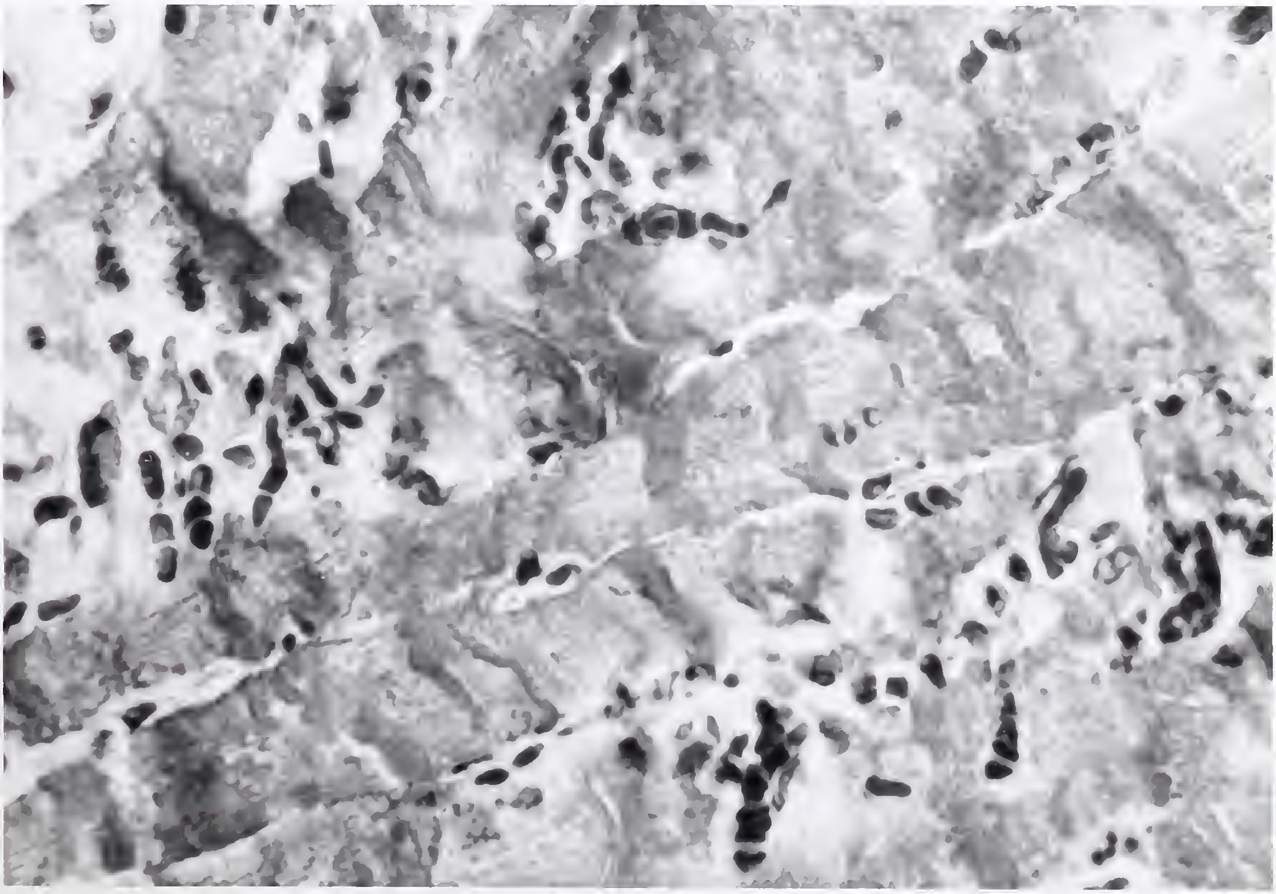


FIGURE 7



C



D

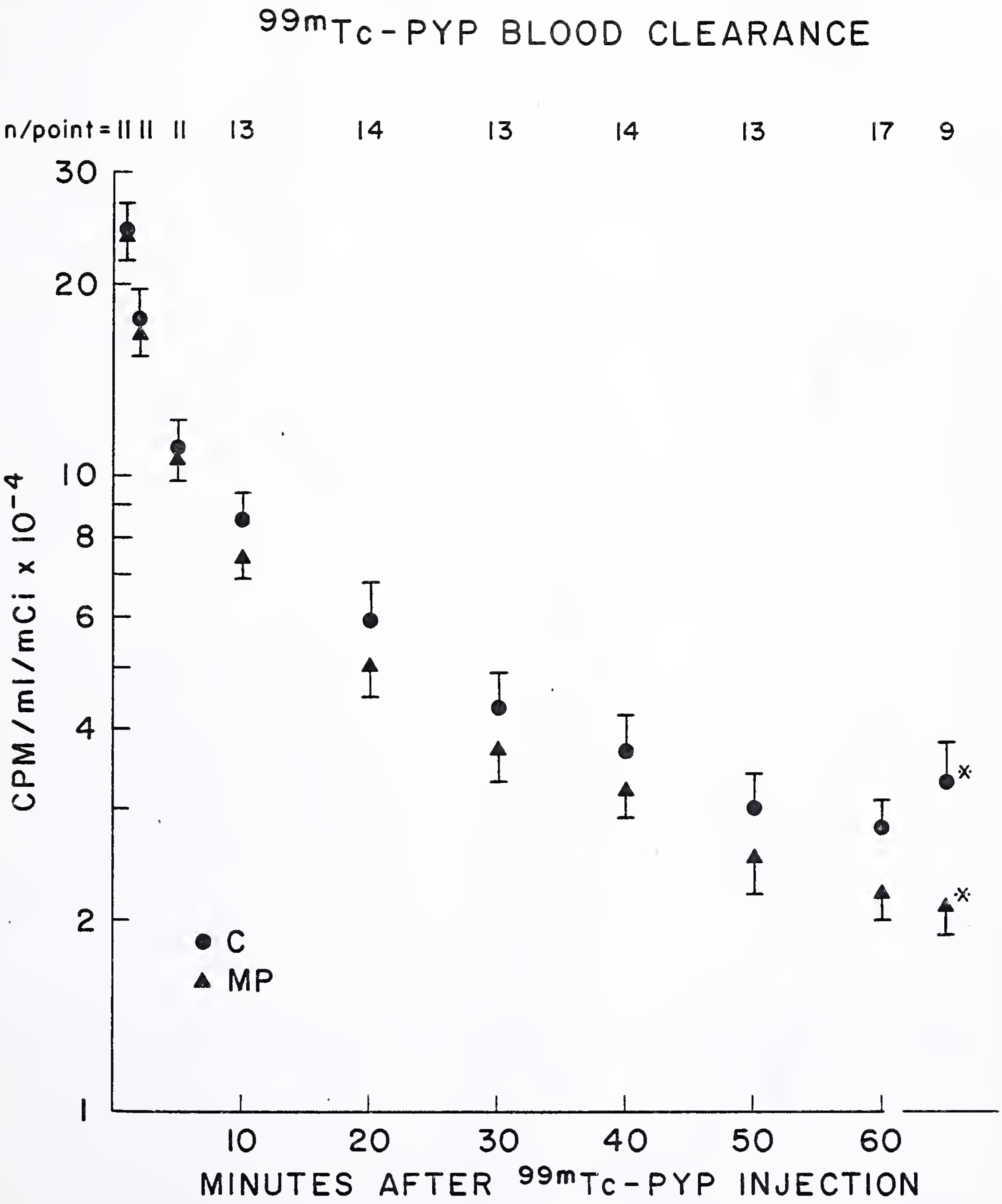

99mTc-PYP BLOOD CLEARANCE
n/point = 11 11 11 13 14 13 14 13 17 9
30
20
10
8
6
4
2
1
CPM/ml/mCi x 10⁻⁴
● C
▲ MP
*
*
10
20
30
40
50
60
MINUTES AFTER 99mTc-PYP INJECTION

FIGURE 8

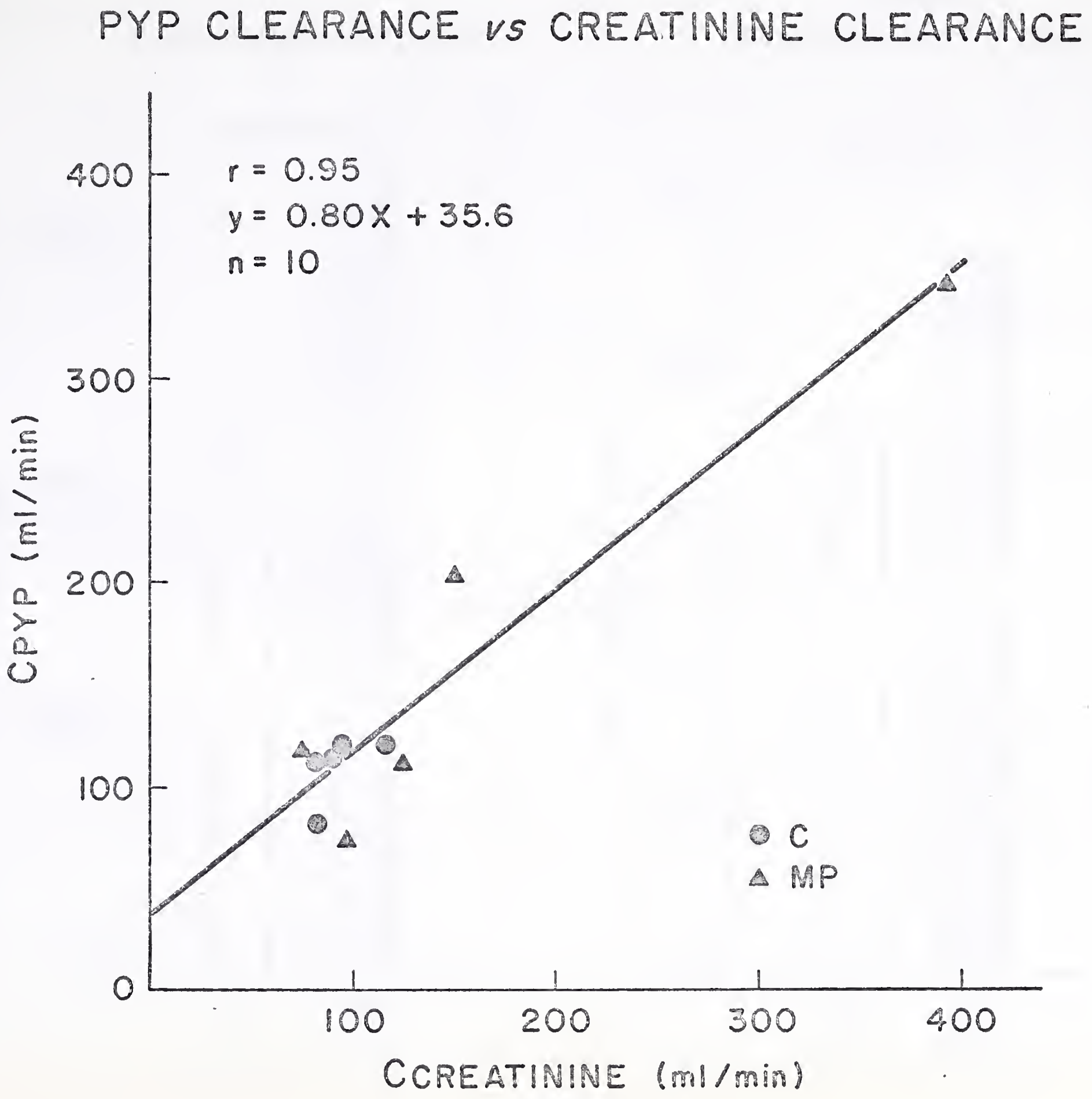

PYP CLEARANCE vs CREATININE CLEARANCE
r = 0.95
y = 0.80X + 35.6
n = 10
CPYP (ml/min)
400
300
200
100
0
100
200
300
400
CCREATININE (ml/min)
● C
▲ MP

FIGURE 9

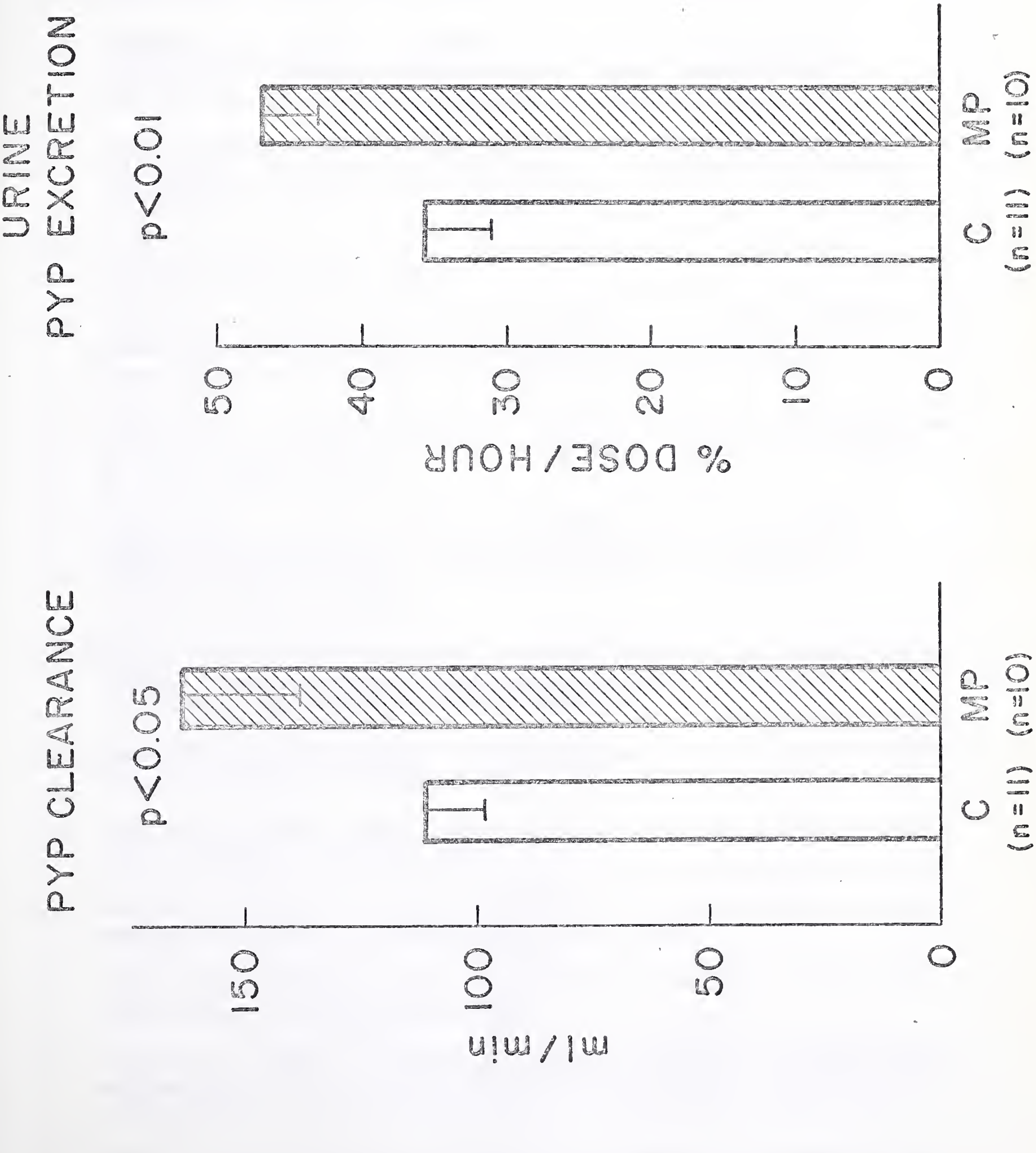

PYP CLEARANCE
p<0.05
ml / min
150
100
50
0
C
MP
(n=11)
(n=10)
URINE
PYP EXCRETION
p<0.01
% DOSE / HOUR
50
40
30
20
10
0
C
MP
(n=11)
(n=10)

FIGURE 10

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